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(54) Title: NOVEL 4-DEDIMETHY LAMINOTETRA CYCLINE DERIVATIVES

(57) Abstract

The present invention provides new chemically modified 4-dedimethylaminotetracycline compounds that can be substituted at the 7,8, and/or 9 positions and methods for preparing the 4-dedimethylamino tetracycline compounds. Other tetracycline compounds are the 4-dedimethylaminotetracycline derivatives with an oxime group, NH-Alkyl, or N-NH-Alkyl group at the C4 position. The present invention also provides a method of treating a mammal suffering from conditions or diseases by administering to the mammal an effective amount of the new chemically modified 4-dedimethylamino tetracycline compounds.

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NOVEL 4-DEDIMETHYLAMINOTETRACYCLINE DERIVATIVES

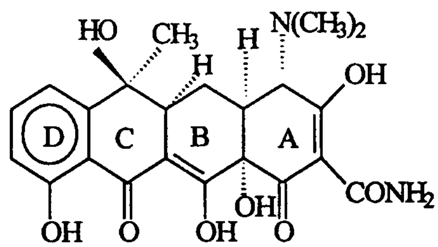
The present application is a continuation-in-part of Provisional U.S. Patent Application Serial No. 60/108,948 filed on November 18, 1998, which is incorporated herein by reference.

FIELD OF INVENTION

The present invention relates to novel 4-dedimethylaminotetracycline derivatives, methods for producing the novel derivatives and methods of using these derivatives.

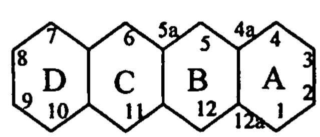
BACKGROUND OF THE INVENTION

The compound, tetracycline, exhibits the following general structure:



Structure A

The numbering system of the ring nucleus is as follows:



Structure B

Tetracycline as well as the 5-OH (Terramycin) and 7-Cl (Aureomycin) derivatives exist in nature, and are well known antibiotics. Natural tetracyclines may be modified without losing their antibiotic properties, although certain elements of the structure must be retained. The modifications that may and may not be made to the basic tetracycline structure have been reviewed by Mitscher in *The Chemistry of Tetracyclines*, Chapter 6, Marcel Dekker, Publishers, New York (1978). According to Mitscher, the substituents at positions 5-9 of the tetracycline ring system may be modified without the complete loss of antibiotic properties. Changes to the basic ring system or replacement of the substituents at positions 1-4 and 10-12, however, generally lead to synthetic tetracyclines with substantially less or effectively no antimicrobial activity. An example of a chemically modified non-antimicrobial tetracycline (hereinafter CMT) is 4-dedimethylaminotetracyline.

Some 4-dedimethylaminotetracyline derivatives are disclosed in U.S. Pat. Nos. 3,029,284 and 5,122,519. They include 6-demethyl-6-deoxy-4-dedimethylaminotetracycline and 5-hydroxy-6-deoxy-4-dedimethylaminotetracycline with hydrogen and other substituents at the C7, and the C9 positions on the D ring. These substituents include amino, nitro, di (lower alkyl) amino, and mono (lower alkyl) amino or halogen. The 6-demethyl-6-deoxy-4-dedimethylaminotetracycline derivatives and 5-hydroxy-6-deoxy-4-dedimethylaminotetracycline derivatives are said to be useful as antimicrobial agents.

Other 4-dedimethylaminotetracycline derivatives with an oxime group at the C4 position on the A ring are disclosed in U.S. Patent Nos. 3,622,627 and 3,824,285. These oxime derivatives have hydrogen and halogen as substituents at the C7 position and include 7-halo-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, and 7-halo-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline.

Alkylamino (NH-alkyl), and alkylhydrazone (N-NH-alkyl) groups have been substituted on the A ring at the C4 position on 4-dedimethylaminotetracycline. These compounds are known for their antimicrobial properties. See U.S. Patent Nos.

3,345,370, 3,609,188, 3,622,627, 3,824,285, 3,622,627, 3,502,660, 3,509,184, 3,502,696, 3,515, 731, 3,265,732, 5,122,519, 3,849,493, 3,772,363, and 3,829,453.

In addition to their antimicrobial properties, tetracyclines have been described as having a number of other uses. For example, tetracyclines are also known to inhibit the activity of collagen destructive enzymes, such as matrix metalloproteinases (MMP), including collagenase (MMP-1), gelatinase (MMP-2) and stromelysin (MMP-3). Golub et al., *J. Periodont. Res.* 20:12-23 (1985); Golub et al. *Crit. Revs. Oral Biol. Med.* 2: 297-322 (1991); U.S. Pat. Nos. 4,666,897; 4,704,383; 4,935,411; 4,935,412. Also, tetracyclines have been known to inhibit wasting and protein degradation in mammalian skeletal muscle, U.S. Pat. No. 5,045,538, and to enhance IL-10 production in mammalian cells.

Furthermore, tetracyclines have been shown to enhance bone protein synthesis in U.S. Pat. No. Re. 34,656. and to reduce bone resorption in organ culture in U.S. Pat. No. 4,704,383.

Similarly, U.S. Pat. No. 5,532,227 to Golub et al. discloses that tetracyclines can ameliorate the excessive glycosylation of proteins. In particular, tetracyclines inhibit the excessive collagen cross linking which results from excessive glycosylation of collagen in diabetes.

Tetracyclines are known to inhibit excessive phospholipase A_2 activity involved in inflammatory conditions such as psoriasis as disclosed in U.S. Pat. No. 5,532,227. In addition, tetracyclines are also known to inhibit cycloxygenase-2 (COX-2), tumor necrosis factor (TNF), nitric oxide and IL-1 (interleukin-1).

These properties cause the tetracyclines to be useful in treating a number of diseases. For example, there have been a number of suggestions that tetracyclines, including non-antimicrobial tetracyclines, are effective in treating arthritis. See, for example, Greenwald, et al. "Tetracyclines Suppress Metalloproteinase Activity in

Adjuvant Arthritis and, in Combination with Flurbiprofen, Ameliorate Bone Damage," *Journal of Rheumatology* 19:927-938(1992); Greenwald et al., "Treatment of Destructive Arthritic Disorders with MMP Inhibitors: Potential Role of Tetracyclines in Inhibition of Matrix Metalloproteinases: *Therapeutic Potential,"* *Annals of the New York Academy of Sciences* 732: 181-198 (1994); Kloppenburg, et al. "Minocycline in Active Rheumatoid Arthritis," *Arthritis Rheum* 37:629-636(1994); Ryan et al., "Potential of Tetracycline to Modify Cartilage Breakdown in Osteoarthritis," *Current Opinion in Rheumatology* 8: 238-247(1996); O'Dell et al, "Treatment of Early Rheumatoid Arthritis with Minocycline or Placebo," *Arthritis Rheum* 40:842-848(1997).

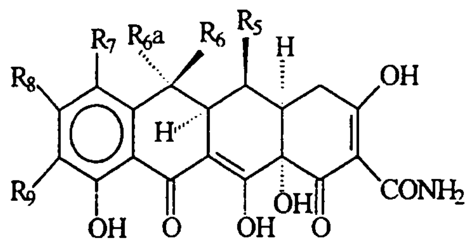
Tetracyclines have also been suggested for use in treating skin diseases. For example, White et al., *Lancet*, Apr. 29, p. 966 (1989) report that the tetracycline minocycline is effective in treating dystrophic epidermolysis bullosa, which is a life-threatening skin condition believed to be related to excess collagenase.

Furthermore, studies have also suggested that tetracyclines and inhibitors of metalloproteinases inhibit tumor progression, DeClerck et al., *Annals N.Y. Acad. Sci.*, 732: 222-232 (1994), bone resorption, Rifkin et al., *Annals N.Y. Acad. Sci.*, 732: 165-180 (1994), angiogenesis, Maragoudakis et al., *Br. J. Pharmacol.*, 111: 894-902 (1994), and may have anti-inflammatory properties, Ramamurthy et al., *Annals N.Y. Acad. Sci.*, 732, 427-430 (1994).

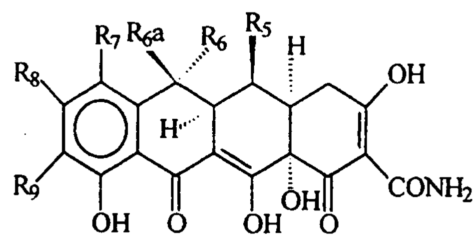
Based on the foregoing, tetracyclines have been found to be effective in different treatments. However, there is a need for new 4-dedimethylaminotetracycline derivatives, methods for producing the novel derivatives and methods of using these derivatives for treatment of different types of diseases or conditions.

SUMMARY OF THE INVENTION

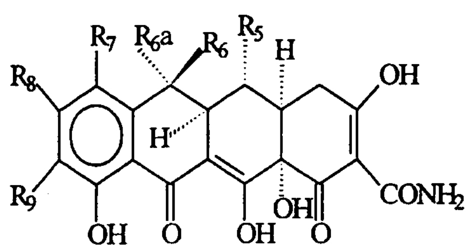
It has now been discovered that these and other objectives can be achieved by a tetracycline compound of the formulae:



Structure C

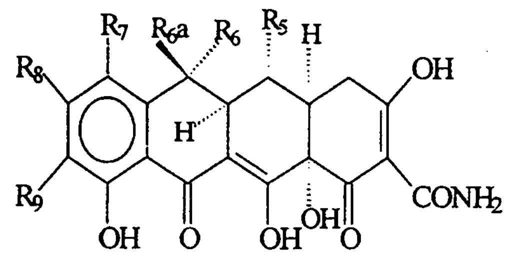


Structure D



Structure E

or

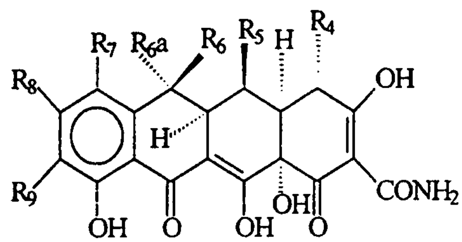


Structure F

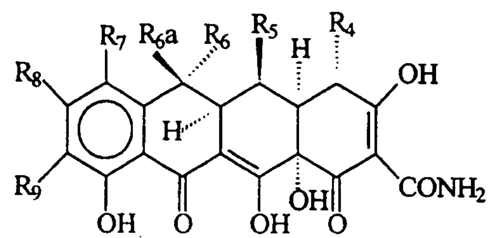
where R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl)amino, halogen, diazonium, di(lower alkyl)amino and $RCH(NH_2)CO$; R is hydrogen or lower alkyl; and pharmaceutically acceptable and unacceptable salts thereof; with the following provisos: when either R7 and R9 are hydrogen then R8 must be halogen; and when

R6-a, R6, R5 and R9 are all hydrogen and R7 is hydrogen, amino, nitro, halogen, dimethylamino or diethylamino, then R8 must be halogen; and when R6-a is methyl, R6 and R9 are both hydrogen, R5 is hydroxyl and R7 is hydrogen, amino, nitro, halogen or diethylamino, then R8 is halogen; and when R6-a is methyl, R6 is hydroxyl, R5, R7 and R9 are all hydrogen, then R8 must be halogen; and when R6-a, R6 and R5 are all hydrogen, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and when R6-a is methyl, R6 is hydrogen, R5 is hydroxyl, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and when R6-a is methyl, R6, R5 and R9 are all hydrogen and R7 is cyano, then R8 must be halogen.

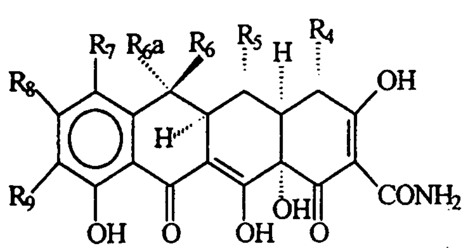
In another embodiment, the invention provides a tetracycline compound of the formulae:



Structure G

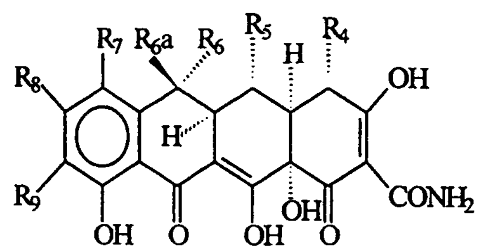


Structure H



Structure I

or



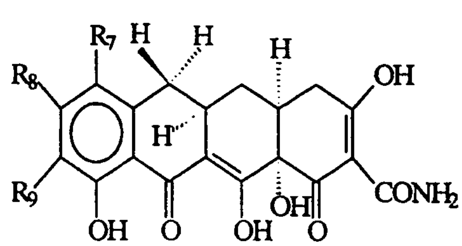
Structure J

where R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group

consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R4 is selected from the group consisting of NOH, N-NH-A, and NH-A, where A is a lower alkyl group; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, di(lower alkyl)amino and $RCH(NH_2)CO$; R is hydrogen or lower alkyl; and pharmaceutically acceptable and unacceptable salts thereof; with the following provisos: when R4 is NOH, N-NH-alkyl or NH-alkyl and R7, R6-a, R6, R5, and R9 are all hydrogen, then R8 must be halogen; and when R4 is NOH, R6-a is methyl, R6 is hydrogen or hydroxyl, R7 is halogen, R5 and R9 are both hydrogen, then R8 must be halogen; and when R4 is N-NH-alkyl, R6-a is methyl, R6 is hydroxyl and R7, R5, R9 are all hydrogen, then R8 must be halogen; and when R4 is NH-alkyl, R6-a, R6, R5 and R9 are all hydrogen, R7 is hydrogen, amino, mono(lower alkyl)amino, halogen, di(lower alkyl)amino or hydroxyl, then R8 must be halogen; and when R4 is NH-alkyl, R6-a is methyl, R6 and R9 are both hydrogen, R5 is hydroxyl, and R7 is mono(lower alkyl)amino or di(lower alkyl)amino, then R8 must be halogen; and when R4 is NH-alkyl, R6-a is methyl, R6 is hydroxy or hydrogen and R7, R5, and R9 are all be hydrogen, then R8 must be halogen.

In yet another embodiment, the invention provides a 4-dedimethylamino tetracycline compound having general formulae (I) through (IV):

General Formula (I)



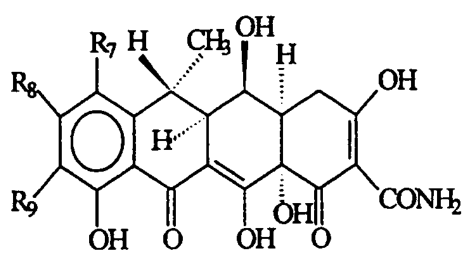
Structure K

where R7, R8, and R9 taken together in each case, have the following meanings:

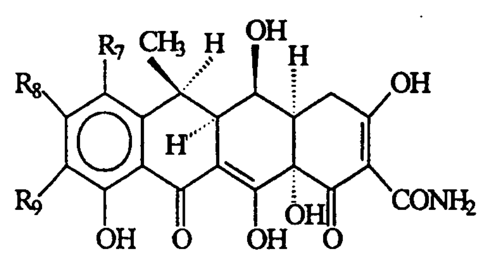
R7	R8	R9
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	acylamino
dimethylamino	hydrogen	diazonium
dimethylamino	chloro	amino
hydrogen	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkylamino	chloro	amino
nitro	chloro	amino
dimethylamino	chloro	acylamino
dimethylamino	chloro	dimethylamino
dimethylamino	hydrogen	hydrogen
hydrogen	hydrogen	dimethylamino

and

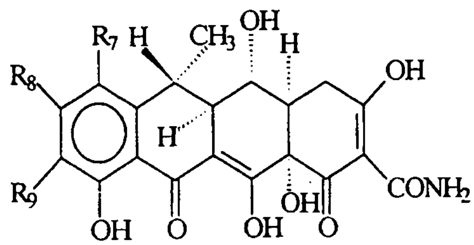
General Formula (II)



Structure L

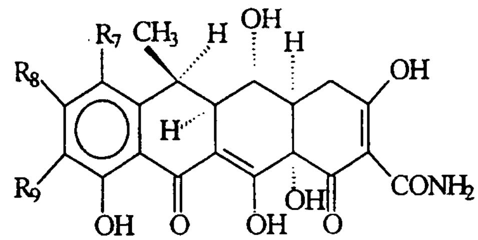


Structure M



Structure N

or



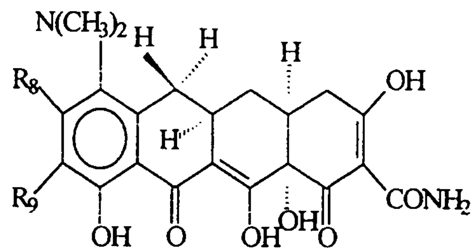
Structure O

where R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	acylamino
hydrogen	hydrogen	diazonium
hydrogen	hydrogen	dimethylamino
diazonium	hydrogen	hydrogen
ethoxythiocarbonylthio	hydrogen	hydrogen
dimethylamino	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
hydrogen	chloro	amino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkyl amino	chloro	amino
nitro	chloro	amino

and

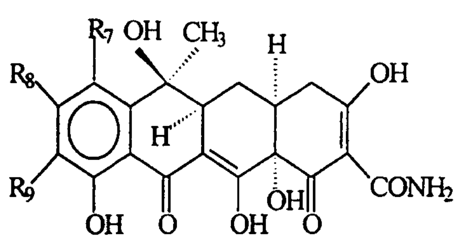
General Formula (III)



Structure P

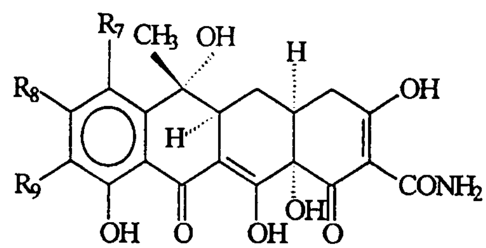
where R8 is hydrogen or halogen and R9 is selected from the group consisting of nitro, (N,N-dimethyl)glycylamino, and ethoxythiocarbonylthio: and

General Formula (IV)



Structure Q

or



Structure R

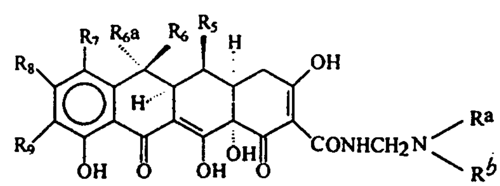
where R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
amino	hydrogen	hydrogen
nitro	hydrogen	hydrogen
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino

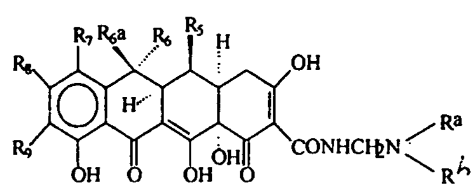
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
bromo	hydrogen	hydrogen
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
diethylamino	hydrogen	hydrogen
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	methylamino
dimethylamino	hydrogen	acylamino
dimethylamino	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
hydrogen	chloro	amino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkylamino	chloro	amino
nitro	chloro	amino

and pharmaceutically acceptable and unacceptable salts thereof.

In yet another embodiment, the invention provides a tetracycline compound of the formulae:



Structure S



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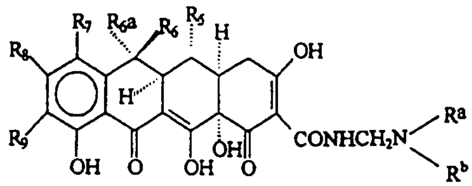

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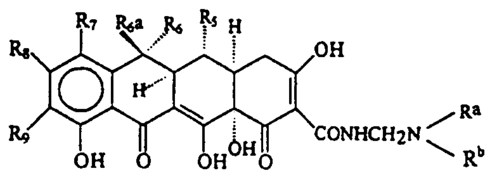

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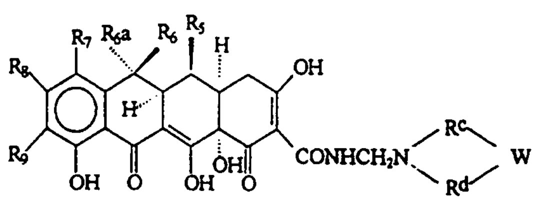
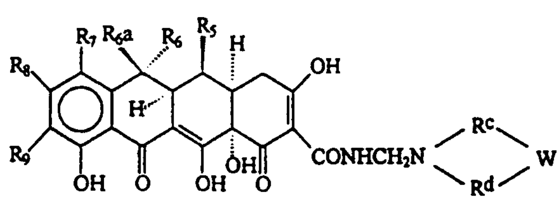
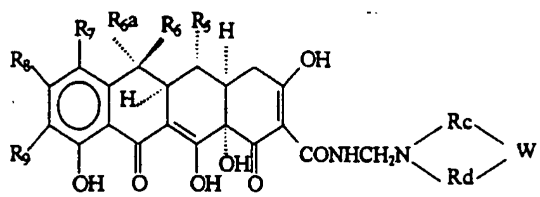


Structure W

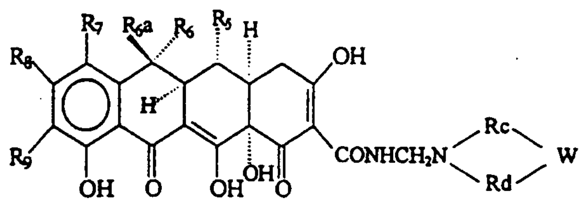


Structure X



Structure Y

or



Structure Z

where R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, diazonium, di(lower alkyl)amino and $RCH(NH_2)CO$; R is hydrogen or lower alkyl; R^a and R^b are selected from the group consisting of hydrogen, methyl, ethyl, n-propyl and 1-methylethyl with the proviso that R^a and R^b cannot both be hydrogen; R^c and R^d are, independently $(CH_2)_nCHR^e$ wherein n is 0 or 1 and R^e is selected from the group consisting of hydrogen, alkyl, hydroxy, lower$(C_1$-$C_3)$ alkoxy, amino, or nitro; and, W is selected from the group consisting of $(CHR^e)_m$ wherein m is 0-3 and R^e is as above, NH, $N(C_1$-$C_3)$ straight chained or branched alkyl, O, S and $N(C_1$-$C_4)$ straight chain or branched alkoxy; and pharmaceutically acceptable and unacceptable salts thereof.

The present invention includes a method for treating a mammal suffering from a condition that benefits from a non-antimicrobial dose of a tetracycline compound. Some examples of such conditions include those characterized by excessive collagen destruction, excessive MMP enzyme activity, excessive TNF activity, excessive nitric oxide activity, excessive IL-1 activity, excessive elastase activity, excessive loss of bone density, excessive protein degradation, excessive muscle wasting, excessive glycosylation of collagen, excessive COX-2 activity, insufficient bone protein synthesis, insufficient interleukin-10 production, or excessive phospholipase A_2 activity. The method for treating comprises administering to the mammal an effective amount of a tetracycline compound of the invention.

These and other advantages of the present invention will be appreciated from the detailed description and examples which are set forth herein. The detailed description and examples enhance the understanding of the invention, but are not intended to limit the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the photoinhibition factor, also known as the photoirritancy factor, (PIF) values for some tetracycline compounds. For structure K, the compounds indicated are as follows:

COL	R7	R8	R9
308	hydrogen	hydrogen	amino
311	hydrogen	hydrogen	palmitamide
306	hydrogen	hydrogen	dimethylamino

For structures L, M, N or O the compounds indicated are as follows:

COL	R7	R8	R9
801	hydrogen	hydrogen	acetamido
802	hydrogen	hydrogen	dimethylaminoacetamido
804	hydrogen	hydrogen	nitro
805	hydrogen	hydrogen	amino

For structure P, R7 is hydrogen, R8 is hydrogen and R9 is nitro.

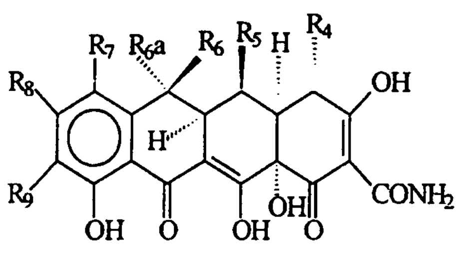
DETAILED DESCRIPTION OF THE INVENTION

Particularly preferred compounds of the present invention have D ring substituents at the 7 and/or 9 positions on the 4-dedimethylaminotetracycline molecule. These compounds include 7-azido-6-demethyl-6-deoxy-4-dedimethylamino tetracycline, 7-dimethylamino-9-azido-6-demethyl-6-deoxy-4-dedimethylamino tetracycline, 9-amino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 9-azido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 9-nitro-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 9-amino-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 7-acetamido-6-demethyl-6-deoxy-4-dedimethylamino tetracycline, 9-acetamido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 7-amino-9-nitro-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 9-(N,N,-dimethyl)glycylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 7, 9-diamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 9-ethoxythiocarbonylthio-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 7-dimethylamino-9-acetamido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 9-azido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 9-azido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 7-azido-5-hydroxy-6-deoxy-4-dedimethylamino tetracycline, 7-dimethylamino-9-azido-5-hydroxy-6-deoxy-4-dedimethylamino tetracycline, 9-amino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 9-azido-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 9-nitro-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 9-amino-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 7-acetamido-5-hydroxy-6-deoxy-4-dedimethylamino tetracycline, 9-acetamido-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 7-amino-9-nitro-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 9-(N,N-dimethyl) glycylamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 7, 9-diamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 7-dimethylamino-9-amino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 9-ethoxythiocarbonylthio-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 7-dimethylamino-9-acetamido-5-

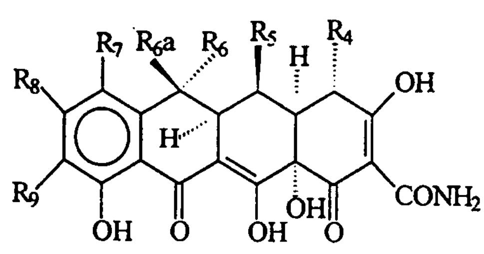
hydroxy-6-deoxy-4-dedimethylaminotetracycline, 9-azido-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 9-amino-8-chloro-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 9-(N,N-dimethyl) glycylamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline, 9-nitro-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline. 9-acetamido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 9-(N, N-dimethyl) glycylamino-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline, and 9-ethoxythiocarbonylthio-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline.

In addition, the D ring may be halogenated at the C8 position to provide 8-halodedimethylaminotetracycline derivatives. As used in this specification, halogens can be chlorine, fluorine, bromine, and iodine. Some examples of 8-halo-dedimethylaminotetracycline derivatives are 9-amino-8-chloro-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline. 9-amino-8-chloro-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline and 9-amino-8-chloro-6-demethyl-6-deoxy-4-dedimethylaminotetracycline.

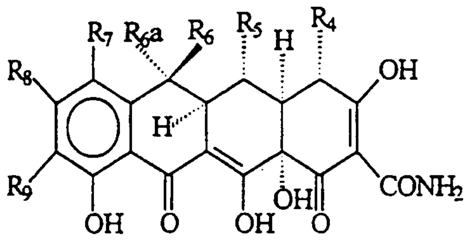
In one embodiment of the invention, the 4-dedimethylaminotetracycline derivatives are substituted with an oxime, NH-alkyl, or N-NH-alkyl group at the C4 position. These compounds have the general formula:



Structure G

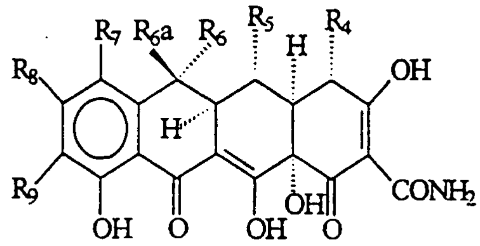


Structure H



Structure I

or



Structure J

wherein R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, and di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R4 is selected from the group consisting of NOH, N-NH-A, and NH-A, where A is a lower alkyl group; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, di(lower alkyl)amino and $RCH(NH_2)CO$; R is hydrogen or lower alkyl; and pharmaceutically acceptable and unacceptable salts thereof; with the following provisos: when R4 is NOH, N-NH-alkyl or NH-alkyl and R7, R6-a, R6, R5, and R9 are all hydrogen, then R8 must be halogen; and when R4 is NOH, R6-a is methyl, R6 is hydrogen or hydroxyl, R7 is halogen, R5 and R9 are both hydrogen, then R8 must be halogen; and when R4 is N-NH-alkyl, R6-a is methyl, R6 is hydroxyl and R7, R5, R9 are all hydrogen, then R8 must be halogen; and when R4 is NH-alkyl, R6-a, R6, R5 and R9 are all hydrogen, R7 is hydrogen, amino, mono(lower alkyl)amino, halogen, di(lower alkyl)amino or hydroxyl, then R8 must be halogen; and when R4 is NH-alkyl, R6-a is methyl, R6 and R9 are both hydrogen, R5 is hydroxyl, and R7 is mono(lower alkyl)amino or di(lower alkyl)amino, then R8 must be halogen; and when R4 is NH-alkyl, R6-a is methyl, R6 is hydroxy or hydrogen and R7, R5, and R9 are all be hydrogen, then R8 must be halogen.

It will be understood that if the stereochemistry of a substituent on rings A-D of the novel 4-dedimethylaminotetracycline derivative is not specified, then both epimers are intended to be encompassed.

As used herein, NH-Alkyl, N-NH-Alkyl, alkoxy and alkyl groups contain straight or branched, saturated or unsaturated alkyl carbon chains, having from one to twenty-six carbon atoms. For example, alkyl groups include fatty alkyls which contain ten to twenty-six carbon atoms. Some examples of saturated fatty alkyl groups include, lauryl, myristyl, palmityl, stearyl, etc. Some examples of unsaturated fatty alkyl groups include palmitoleyl, oleyl, linoleyl, linolenyl, etc.

Alkyl groups also include lower alkyls which include straight or branched, saturated or unsaturated carbon chains, having from one to six carbon atoms. Some examples of lower alkyl groups are methyl, ethyl, propyl, butyl, isobutyl, n-butyl, secondary butyl, tertiary butyl, n-pentyl and benzyl. The alkyl moiety of acyl groups is defined as above. Some examples of acyl groups include acetyl, propionyl, butyryl, and acyl groups comprising fatty acids such as those described above.

Preferred oxime compounds include 7-azido-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-dimethylamino-9-azido-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-amino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-azido-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-nitro-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-amino-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-acetamido-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-acetamido-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-amino-9-nitro-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-(N,N,-dimethyl)glycylamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7, 9-diamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-ethoxythiocarbonylthio-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-dimethylamino-9-

acetamido-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-azido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-azido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-azido-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-dimethylamino-9-azido-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximino-tetracycline, 9-amino-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-azido-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline,

9-nitro-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-amino-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-acetamido-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-acetamido-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-amino-9-nitro-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-(N,N-dimethyl)glycylamino-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, also known as 9-dimethylaminoacetamido-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7, 9-diamino-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-dimethylamino-9-amino-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-ethoxythiocarbonylthio-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 7-dimethylamino-9-acetamido-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-azido-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-amino-8-chloro-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-(N,N-dimethyl) glycylamino-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-nitro-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-acetamido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximino- tetracycline, 9-(N, N-dimethyl) glycylamino-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximino-tetracycline, and 9-ethoxythiocarbonylthio-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline.

In addition, the D ring may be halogenated at the C8 position to provide 8-halo-4-dedimethylamino-4-oximinotetracycline compounds. Some examples include 9-amino-8-chloro-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline, 9-amino-8-chloro-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylamino-4-oximinotetracycline and 9-amino-8-chloro-6-demethyl-6-deoxy-4-dedimethylamino-4-oximinotetracycline.

Preferred hydrazone compounds include 7-azido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-dimethylamino-9-azido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-amino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-azido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-nitro-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-amino-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-acetamido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-acetamido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-amino-9-nitro-6-demethyl-6-deoxy-4-dedimethylaminotetracycline -4-methyl or ethyl hydrazone, 9-(N,N,-dimethyl)glycylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7, 9-diamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-ethoxythiocarbonylthio-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-dimethylamino-9-acetamido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-azido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino tetracycline -4-methyl or ethyl hydrazone, 9-azido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-azido-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-dimethylamino-9-azido-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-amino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-azido-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-

nitro-5-hydroxy-6-deoxy-4-dedimethylamino tetracycline-4-methyl or ethyl hydrazone, 9-amino-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-acetamido-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-acetamido-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-amino-9-nitro-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-(N,N-dimethyl) glycylamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7, 9-diamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-dimethylamino-9-amino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-ethoxythiocarbonylthio-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 7-dimethylamino-9-acetamido-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-azido-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-amino-8-chloro-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-(N,N-dimethyl) glycylamino-5-hydroxy-6-deoxy-4-dedimethylamino tetracycline-4-methyl or ethyl hydrazone, 9-nitro-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-acetamido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, 9-(N, N-dimethyl) glycylamino-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone, and 9-ethoxythiocarbonylthio-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone.

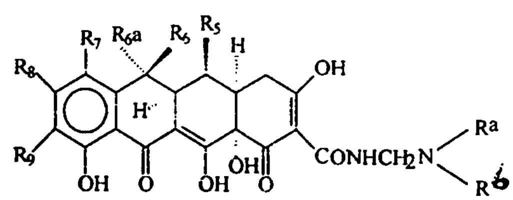
The D ring may be halogenated at the C8 position to provide 8-halo-4-dedimethylaminotetracycline-4-hydrazone compounds. Some examples include 9-amino-8-chloro-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino tetracycline -4-methyl or ethyl hydrazone, 9-amino-8-chloro-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylamino tetracycline-4-methyl or ethyl hydrazone and

9-amino-8-chloro-6-demethyl-6-deoxy-4-dedimethylaminotetracycline-4-methyl or ethyl hydrazone.

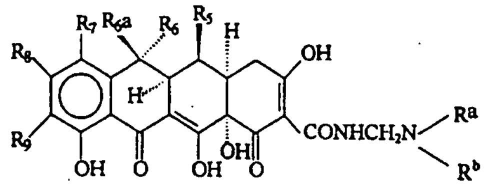
Novel 4-dedimethlyaminotetracycline derivatives of the present invention also include compounds with an NH-Alkyl (alkylamino) substituent at the C4 position on the A ring. These compounds have substitutions at the C5, C6, C6a, C7, C8 and/or C9 positions as described above. An example is 9-azido-8-chloro-7-dimethylamino-5-hydroxy-6-deoxy-4-dedimethylaminotetracycline.

In addition, a hexanoylamino group can be added to the C9 position on the D ring of any compound of the invention. An example includes, but is not limited to, 4-dedimethylamino-6-demethyl-6-deoxy-9-hexanoylaminotetracycline.

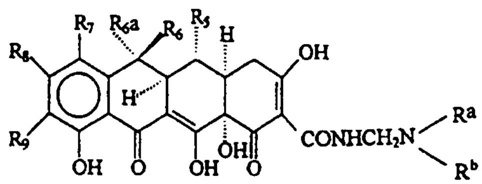
In another embodiment of the invention, the 4-dedimethylaminotetracycline derivatives are Mannich derivatives of the compounds described above. Such derivatives include, for example, compounds having the general formula:



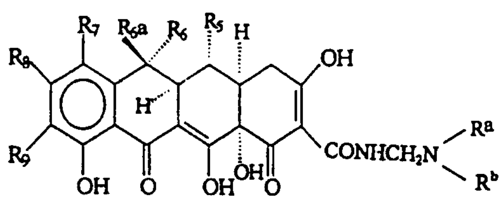
Structure S



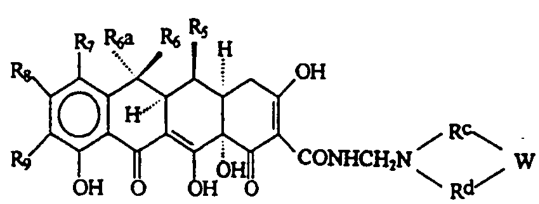
Structure T



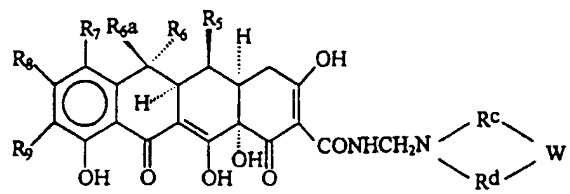
Structure U



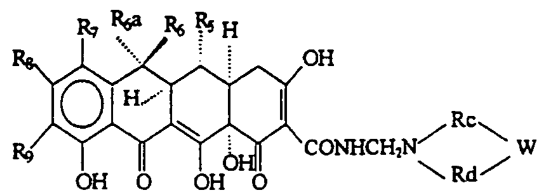
Structure V



Structure W

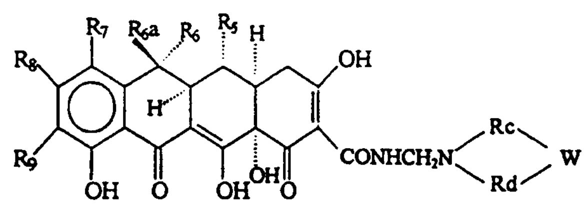


Structure X



Structure Y

or



Structure Z

wherein R5, R6, R7, R8 and R9 are as described above; R^a and R^b are selected from the group consisting of hydrogen, methyl, ethyl, n-propyl or 1-methylethyl with the proviso that R^a and R^b cannot both be hydrogen; R^c and R^d are, independently, $(CH_2)_nCHR^e$ wherein n is 0 or 1 and R^e is selected from the group consisting of

hydrogen, alkyl, hydroxy, lower(C_1-C_3) alkoxy, amino, or nitro; and, W is selected from the group consisting of $(CHR^e)_m$ wherein m is 0-3 and R^e is as above, NH, N(C_1-C_3) straight chained or branched alkyl, O, S and N(C_1-C_4) straight chain or branched alkoxy; and pharmaceutically acceptable and unacceptable salts thereof. For example, when m is 0, R^c and R^d are bonded to each other in a 3-5 membered ring, such as, for example, a pyrrolidino or substituted pyrrolidino ring, a morpholino or substituted morpholino ring, or a piperazino or substituted piperazino ring.

These Mannich derivatives include, for example, compounds with a piperazin-1-yl, 4-methylpiperazin-1-yl, morpholin-1-yl, or pyrrolidin-1-yl substituent at the C2 position. These compounds have substituents at the C4, C5, C6, C6a, C7, C8 and/or C9 positions as described above. Examples of such compounds include, but are not limited to, N-morpholin-1-ylmethyl-4-dedimethylamino-6-demethyl-6-deoxytetracyline, N-pyrrolidin-1-ylmethyl-4-dedimethylamino-6-demethyl-6-deoxytetracyline, N-morpholin-1-ylmethyl-4-dedimethylamino-6-demethyl-6-deoxy-9-hexanoylaminotertracycline, N-pyrrolidin-1-ylmethyl-4-dedimethylamino-6-demethyl-6-deoxy-9-hexanoylaminotertracycline.

The novel 4-dedimethylaminotetracycline compounds of the present invention or salts thereof may be prepared by D ring substitution at the C7, C8 and/or C9 positions using starting reactants that can readily be prepared or purchased by methods known in the art. See, for example, Mitscher, L.A., The Chemistry of the Tetracycline Antibiotics, Marcel Dekker, New York (1978), Ch. 6, Hlavka, J. and J.H. Boothe, The Tetracyclines, Springer-Verlag, Berlin-Heidelberg, page 18 (1985) and U.S. Pat. Nos. 4,704,383, 3,226,436, 3,047,626 3,518,306 and 5,532,227.

For example, nitration of the C9 position on the D ring may be accomplished, and novel 9-nitro compounds may be prepared, by using known starting reactants such as 7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline or 6-deoxy-4-dedimethylaminotetracycline and treating these compounds with a strong acid and metal nitrate salts. Examples of strong acids that are suitable for use in the

present invention are: sulfuric acid, trifluoroacetic acid, methanesulfonic acid or perchloric acid. Suitable metal nitrate salts are, for example, calcium, potassium or sodium nitrate. The C9 position on the D ring undergoes nitration to form the corresponding 9-nitro-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline or 9-nitro-6-deoxy-4-dedimethylaminotetracycline compounds.

Amination of the C9 position on the D ring may be accomplished by treating a 9-nitro-4-dedimethylaminotetracycline, such as 9-nitro-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline or 9-nitro-6-deoxy-4-dedimethylaminotetracycline with hydrogen in the presence of a suitable supported catalyst such as Raney nickel, platinum oxide or palladium-on-carbon. This is then filtered and washed with an organic solvent such as ether. The C9 substituant is reduced to form the corresponding 9-amino-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline or 9-amino-6-deoxy-4-dedimethylaminotetracycline compound.

The amino group on the D ring at the C9 position may be converted to an acylamido group, preferably a sulfonamido group. For example, 9-amino-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline or 9-amino-6-deoxy-4-dedimethylaminotetracycline compounds are treated with acyl chloride, acyl anhydride, mixed acyl anhydride, sulfonyl chloride or sulfonyl anhydride in the presence of a suitable acid scavenger dispersed in a solvent. The acid scavenger is suitably selected from sodium bicarbonate, sodium acetate, pyridine, triethylamine, N,O-bis(trimethylsilyl)acetamide, N,O-bis(trimethylsilyl)trifluoroacetamide or a basic ion-exchange resin. Solvents suitable for the acylation reaction include water, water-tetrahydrofuran, N-methylpyrolidone, 1,3-dimethyl-2-imidazolidione, hexamethylphosphoramide, 1,3,dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone or 1,2-dimethoxyethane. The C9 amino group may be converted to the acetamido group to form, for example, the corresponding 9-acetamido-7-dimethylamino-6-demethyl-6-

deoxy-4-dedimethylamino tetracycline or 9-acetamido-6-deoxy-4-dedimethylamino tetracycline.

A diazonium group can also be substituted at the C9 position on the D-ring. Typically, a 9-amino-4-dedimethylaminotetracycline derivative, such as 9-amino-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline or 9-amino-6-deoxy-4-dedimethylaminotetracycline in 0.1N HCL in methanol is treated with n-butyl nitrite to form the corresponding 9-diazonium derivatives such as 9-diazonium-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline or 9-diazonium-6-deoxy-4-dedimethylaminotetracycline.

The 9-diazonium-4-dedimethylaminotetracycline derivatives, such as 9-diazonium-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline or 9-diazonium-6-deoxy-4-dedimethylaminotetracycline can be treated with methanolic hydrochloric acid plus a triazo compound such as sodium azide to form, 9-azido derivatives, such as 9-azido-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline or 9-azido-6-deoxy-4-dedimethylaminotetracycline.

Alternately, an ethoxythiocarbonylthio group can be substituted at the C9 position on the D ring. For example, a 9-diazonium-4-dedimethylaminotetracycline derivative, such as 9-diazonium-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino tetracycline or 9-diazonium-6-deoxy-4-dedimethylaminotetracycline is treated with an acid metal salt such as potassium ethyl xanthate to form the corresponding 9-ethoxythiocarbonylthio derivative, such as 9-ethoxythiocarbonylthio-7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylamino or 9-ethoxythiocarbonylthio-6-deoxy-4-dedimethylaminotetracycline.

The above reactions describe substitution at the C9 position on 4-dedimethylaminotetracycline molecule. Some substitution may also occur, depending on the starting reactants and conditions used, at the C7 position and lead to also 7-substituted-4-dedimethylaminotetracycline derivatives, such as 7-diazonium-6-

demethyl-6-deoxy-4-dedimethylaminotetracycline or 7-azido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline. The 7-substituted derivatives can be separated from the 9-substituted derivatives, and purified as discussed below.

The novel 7 or 9 azido-4-dedimethylamino derivatives of the present invention can be halogenated at the C8 position by treating 7 or 9-azido-4-dedimethylaminotetracycline with a strong acid such as hydrogen halide, sulfuric acid saturated with hydrogen halide or methanesulfonic acid saturated with hydrogen halide. The product that is isolated, when the hydrogen halide is hydrogen chloride, is the 8-chloro (7 or 9) amino-4-dedimethylaminotetracycline derivative. A particularly preferred halogenated compound is 9-acetamido-8-chloro-7-dimethylamino-6-deoxy-6-demethyl-4-dedimethylamino tetracycline.

In one embodiment, 4-dedimethylaminotetracycline compounds of the present invention have an oxime (NOH), alkylamino (NH-alkyl), or alkylhydrazone (N-NH-alkyl) group at the C4 position on the A ring. These compounds can be made using known methods. For example, 4-hydroxytetracycloxide may be treated with hydroxyamine or ethylhydrazine under alkaline conditions in a solvent such as methanol or ethanol. Substitution at the C4 position occurs and 4-dedimethylamino-4-oximinotetracycline and 4-dedimethylaminotetracycline-4-alkylhydrazone compounds can be isolated as alkali metal salts. See for example, U.S. Patent Nos. 3,622,627, 3,159,675 and 3,345,370. Substitution at C7, C8, and/or C9 positions on the D ring using methods previously described (i.e. halogenation, amination, or nitration) give rise to the novel 4-oxime, 4-hydrazone and 4-aminoalkyl compounds of the present invention.

The Mannich derivatives can be made by methods known in the art. For example, the tetracycline derivatives described above may be treated with formaldehyde and the appropriate amine.

Examples of specific embodiments are described above as derivatives of tetracycline. The compounds of the invention are not, however, limited to tetracycline derivatives. The invention also includes, but is not limited to, the same 4-dedimethylamino derivatives and 4-substituted 4-dedimethylamino derivatives of sancycline, minocycline, and doxycycline as the tetracycline derivatives mentioned above.

The present invention embraces salts, including acid-addition and metal salts, of the 4-dedimethylaminotetracycline compounds. Such salts are formed by well known procedures with both pharmaceutically acceptable and pharmaceutically unacceptable acids and metals. By "pharmaceutically acceptable" is meant those salt-forming acids and metals which do not substantially increase the toxicity of the compound.

Some examples of suitable salts include salts of mineral acids such as hydrochloric, hydriodic, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, as well as salts of organic acids such as tartaric, acetic, citric, malic, benzoic, glycollic, gluconic, gulonic, succinic, arylsulfonic, e.g. p-toluenesulfonic acids, and the like. The pharmaceutically unacceptable acid addition salts, while not useful for therapy, are valuable for isolation and purification of the new substances. Further, they are useful for the preparation of pharmaceutically acceptable salts. Of this group, the more common salts include those formed with hydrofluoric and perchloric acids. Hydrofluoride salts are particularly useful for the preparation of the pharmaceutically acceptable salts, e.g. the hydrochlorides, by dissolution in hydrochloric acid and crystallization of the hydrochloride salt formed. The perchloric acid salts are useful for purification and crystallization of the new products.

Whereas metal salts may, in general, be prepared and are useful for various purposes, the pharmaceutically acceptable metal salts are particularly valuable because of their utility in therapy. The pharmaceutically acceptable metals include more commonly sodium, potassium and alkaline earth metals of atomic number up to

and including 20, i.e., magnesium and calcium and additionally, aluminum, zinc, iron and manganese, among others. Of course, the metal salts include complex salts, i.e. metal chelates, which are well recognized in the tetracycline art.

After preparation, the novel compounds of the present invention can be conveniently purified by standard methods known in the art. Some suitable examples include crystallization from a suitable solvent or partition-column chromatography.

The novel 4-dedimethylaminotetracycline compounds of the present invention can be used *in vivo, in vitro,* and *ex vivo,* for example, in living mammals as well as in cultured tissue, organ or cellular systems. Mammals include, for example, humans, as well as pet animals such as dogs and cats, laboratory animals, such as rats and mice, and farm animals, such as horses and cows. Tissues, as used herein, are an aggregation of similarly specialized cells which together perform certain special functions. Cultured cellular systems include any mammalian cells, such as epithelial, endothelial, red blood, and white blood cells. More particularly, human peripheral blood monocytes, synovial fibroblastoid cells, and the like.

The present invention is directed to a method for treating a mammal suffering from a condition or diseases that benefits from a non-antimicrobial dose of a tetracycline compound. These conditions or diseases are characterized by excessive collagen destruction, excessive MMP enzyme activity, excessive TNF activity, excessive nitric oxide activity, excessive IL-1 activity, excessive elastase activity, excessive loss of bone density, excessive protein degradation, excessive muscle wasting, excessive glycosylation of collagen, excessive COX-2 activity, insufficient bone protein synthesis, insufficient IL-10 (interleukin-10) production or excessive phospholipase A_2 activity. The method comprises administering to the mammal an effective amount of a tetracycline compound of the invention.

The term "excessive," as used herein, refers to increased activity over usual activity which leads to some pathological problem in a mammal or mammalian cells.

In vivo practice of the invention permits application in the relief or palliation of medical and veterinary diseases, conditions, and syndromes. In particular, the present invention includes a method for treating a mammal suffering from conditions or diseases including abdominal aortic aneurysm, ulceration of the cornea, periodontal disease, diabetes, diabetes mellitus. scleroderma. progeria, lung disease, cancer, graft versus host diseases, disease of depressed bone marrow function, thrombocytopenia, prosthetic joint loosening, spondyloarthropathies. osteoporosis, Paget's disease, autoimmune disease, systemic lupus erythematosus, acute or chronic inflammatory conditions, renal disease or connective tissue disease by administering an effective amount of a tetracycline compound to the mammal.

Cancerous conditions treatable by tetracycline compounds of the present invention include, but are not limited to. carcinomas, blastomas, sarcomas such as Kaposi Sarcoma, glioma, and the twelve major cancers: prostrate cancer, breast cancer, lung cancer, colorectal cancer. bladder cancer, non-Hodgkin's lymphoma, uterine cancer, melanoma, kidney cancer, leukemia, ovarian cancer and pancreatic cancer.

Acute or chronic inflammatory conditions treatable by tetracycline compounds of the present invention include, for example, inflammatory bowel disease, arthritis, osteoarthritis, rheumatoid arthritis, pancreatitis, nephritis, glomerulonephritis. sepsis, septic shock, lipopolysaccharide endotoxin shock, multisystem organ failure or psoriasis.

Lung diseases treatable by means of the present invention include, for example, ARDS (adult respiratory distress syndrome), cystic fibrosis, emphysema or acute lung injury resulting from inhalation of toxicants. Some examples of toxicants are acids, chemicals, industrial and military poisons, smoke and other toxic products of combustion.

The novel tetracycline compounds of the present invention can also be used to treat renal diseases. Some examples of renal diseases are chronic renal failure, acute renal failure, nephritis or glomerulonephritis.

An effective amount of a tetracycline compound as used herein is that amount effective to achieve the specified result of treating the disease or condition. Preferably, the tetracycline compound or derivative is provided in an amount which has little or no antimicrobial activity. A tetracycline compound or derivative is not effectively antimicrobial if it does not significantly prevent the growth of microbes. Accordingly, the method can beneficially employ a tetracycline derivative which has been modified chemically to reduce or eliminate its antimicrobial properties. The use of such chemically-modified tetracyclines is preferred in the present invention since they can be used at higher levels than antimicrobial tetracyclines, while avoiding certain disadvantages, such as the indiscriminate killing of beneficial microbes, and the emergence of resistant microbes, which often accompanies the use of antimicrobial or antibacterial amounts of such compounds.

The maximal dosage for a mammal is the highest dosage which does not cause undesirable or intolerable side effects. Minimal dosage is the lowest dosage where efficacy is first observed. For example, the tetracycline compound can be administered in an amount of from about 0.1 mg/kg/day to about 30 mg/kg/day, and preferably from about 1 mg/kg/day to about 18 mg/kg/day. In any event, the practitioner is guided by skill and knowledge in the field, and the present invention includes without limitation dosages which are effective to achieve the described effect.

The method involves administering or providing a tetracycline derivative in an amount which is effective for treating diseases or conditions in mammalian cells or a mammal. Administering the tetracycline derivatives can be accomplished in a variety of ways. In cultured cellular systems (*in vitro*), tetracycline derivatives can be

administered by contacting the cells directly with an effective amount of the tetracycline derivative.

In living mammals (*in vivo*), tetracycline derivatives of the present invention can be administered systemically by the parenteral and enteral routes which also includes controlled release delivery systems. For example, tetracycline derivatives of the present invention can easily be administered intravenously (e.g., intravenous injection) which is a preferred route of delivery. Intravenous administration can be accomplished by mixing the tetracycline derivatives in a suitable pharmaceutical carrier (vehicle) or excipient as understood by practitioners in the art.

Oral or enteral use is also contemplated, and formulations such as tablets, capsules, pills, troches, elixirs, suspensions, syrups, wafers, chewing gum and the like can be employed to provide the tetracycline derivative.

Alternatively, delivery of the tetracycline derivative can include topical application. Accordingly, the carrier is preferably suited for topical use. Compositions deemed to be suited for such topical use include gels, salves, lotions, creams ointments and the like. The tetracycline derivative may also be incorporated with a support base or matrix or the like to provide a pre-packaged surgical or burn dressing or bandage which can be directly applied to skin. Topical application of tetracycline derivatives in amounts of up to about 25% (w/w) in a vehicle are therefore appropriate depending upon indication. More preferably, application of tetracycline derivatives in amounts of from about 0.1% to about 10% is believed to be effective in treating diseases or conditions. It is believed that these quantities do not induce significant toxicity in the subject being treated.

For example, in certain cases tetracycline compounds having only limited biodistribution may be preferred for localized activity. Topical application of these non-absorbable CMTs would be desirable in oral lesions, since the CMTs would not be absorbed to any significant degree even if swallowed.

Combined or coordinated topical and systemic administration of tetracycline derivatives is also contemplated under the invention. For example, a non-absorbable tetracycline compound can be administered topically, while a tetracycline compound capable of substantial absorption and effective systemic distribution in a subject can be administered systemically.

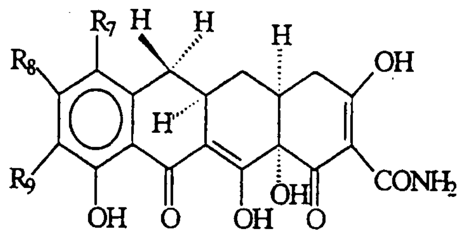
PHOTOTOXICITY

In one embodiment, the invention relates to a class of compounds that have low phototoxicity. To identify potentially phototoxic tetracycline derivatives, the 3T3 Neutral Red Phototoxicity assay was employed. The assay is described in Toxicology In Vitro 12:305-327, 1998.

Briefly, 3T3 cells are seeded in to 96-well plates and incubated over night. The growth medium is removed and replaced with phenol-red free Hanks' Balanced Salt Solution containing serial dilutions of the CMTs (two plates per compound). After an initial one hour incubation at 37^0C, one plate is exposed to 5 Joules/cm^2 of UVA/white light from a solar simulator while the other is held in the dark. The plates are then rinsed, refed and incubated for 24 hours. Cell visibility is measured by neutral red uptake. Phototoxicity is measured by the relative toxicity between the doses with and without light exposure following published guidelines. (Reference compounds include commercially available tetracycline. doxycycline, and minocycline.) The relative phototoxicity is called photoinhibition factor (PIF). The phototoxic response of the compounds in the present assay is consistent with their behavior in vivo.

The class of low phototoxicity tetracyline derivatives has less than 75% of the phototoxicity of minocycline, preferably less than 70%, more preferably less than 60%, and most preferably 50% or less. Optimally, the class of low phototoxicity tetracycline derivatives have PIF values of 1. At a PIF value of 1, a compound is considered to have no measurable phototoxicity. Members of this class include, but

are not limited to, tetracycline compounds, as shown in Figure 1, having general forumulae:

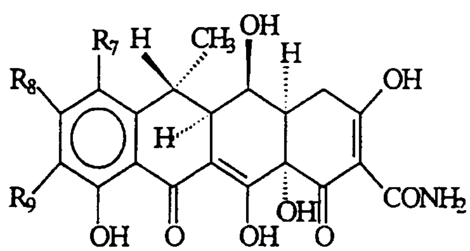


Structure K

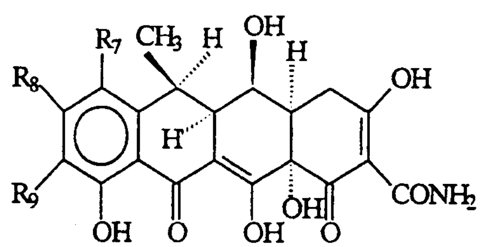
wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	amino
hydrogen	hydrogen	palmitamide
hydrogen	hydrogen	dimethylamino

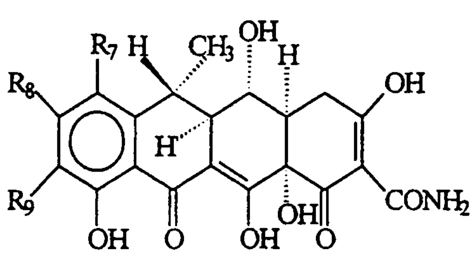
and



Structure L

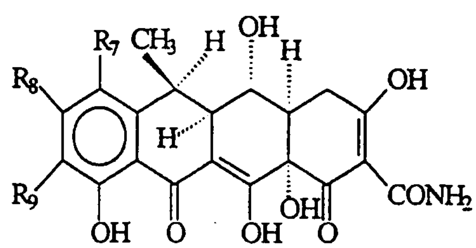


Structure M



Structure N

or

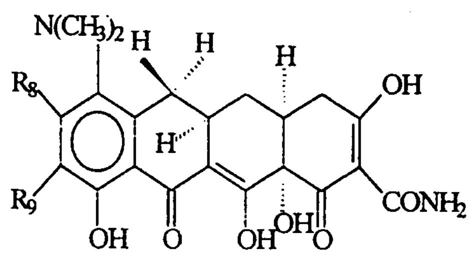


Structure O

wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	acetamido
hydrogen	hydrogen	dimethylaminoacetamido
hydrogen	hydrogen	nitro
hydrogen	hydrogen	amino

and



Structure P

wherein: R7, R8, and R9 taken together are, respectively, hydrogen, hydrogen and nitro.

EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention.

EXAMPLE 1

4-Dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-nitrotetracycline sulfate

To a solution of one millimole of 4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline in 25 ml of concentrated sulfuric acid at 0°C was added 1.05 mmole of potassium nitrate. The resulting solution was stirred at ice bath temperature for 15 minutes and poured in one liter of cold ether with stirring. The precipitated solid was allowed to settle and the majority of solvent decanted. The remaining material was filtered through a sintered glass funnel and the collected solid was washed well with cold ether. The product was dried in a vacuum desiccator overnight.

EXAMPLE 2

9-amino-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline sulfate

To a solution of 300 mg of the 9-nitro compound from example 1, in 30 ml of ethanol was added 50 mg of PtO_2. The mixture was hydrogenated at atmospheric pressure until the theoretical amount of hydrogen was absorbed. The system is flushed with nitrogen, the catalyst PtO_2 is filtered and the filtrate added dropwise to 300 ml of ether. The product that separates is filtered and dried in a vacuum desiccator.

EXAMPLE 3

9-Acetamido-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline

sulfate

To a well stirred cold solution of 500 mg of 9-amino-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline sulfate from example 2, in 2.0 ml of 1,3-dimethyl-2-imidazolidinone, 500 mg of sodium bicarbonate was added followed by 0.21 ml of acetyl chloride. The mixture is stirred at room temperature for 30 minutes, filtered and the filtrate was added dropwise to 500 ml of ether. The product that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 4

4-Dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-diazoniumtetracycline sulfate

To a solution of 0.5 g of 9-amino-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline sulfate, from example 2, in 10 ml of 0.1N hydrochloric acid in methanol cooled in an ice bath, 0.5 ml of n-butyl nitrite was added. The solution was stirred at ice bath temperature for 30 minutes and then poured into 250 ml of ether. The product that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 5

9-Azido-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline sulfate

To a solution of 0.3 mmole of 4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-diazoniumtetracycline sulfate, from example 4, 10 ml of 0.1 N methanolic hydrogen chloride was added 0.33 mmole of sodium azide. The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then poured

into 200 ml of ether. The product that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 6

9-Amino-8-chloro-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-tetracycline sulfate

One gram of 9-azido-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline hydrochloride, from example 4, was dissolved in 10 ml of concentrated sulfuric acid saturated with HCL at 0°C. The mixture was stirred at ice bath temperature for 1.5 hours and then slowly added dropwise to 500 ml of cold ether. The product that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 7

4-Dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-ethoxythiocarbonylthio-tetracycline sulfate

A solution of 1.0 mmole of 4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-diazoniumtetracycline sulfate, from example 4, in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The product separated and was filtered and dried in a vacuum desiccator.

EXAMPLE 8A

General Procedure for Nitration

To 1 mmole of a 4-dedimethylamino-6-deoxytetracycline in 25 ml of concentrated sulfuric acid at 0°C was added 1 mmole of potassium nitrate with stirring. The reaction solution was stirred for 15 minutes and then poured into 100 g of chopped ice. The aqueous solution was extracted 5 times with 20 ml of butanol

each time. The butanol extracts were washed three times with 10 ml of water each time, and concentrated *in vacuo* to a volume of 25 ml. The light yellow crystalline solid which precipitated was filtered, washed with 2 ml of butanol and dried *in vacuo* at 60°C for 2 hours. This solid was a mixture of the two mononitro isomers.

EXAMPLE 8B

4-Dedimethylamino-6-deoxy-9-nitrotetracycline

To 980 mg of the nitration product from 4-dedimethylamino-6-deoxytetracycline (a mixture of the 2 isomers) in 25 ml of methanol was added enough triethylamine to dissolve the solid. The filtered solution (pH 9.0) was adjusted to pH 5.2 with concentrated sulfuric acid. A crystalline yellow solid (236 mg.) was obtained (29% yield). The material at this point was quite pure and contained only small amounts of the 7-isomer. Final purification was accomplished by liquid partition chromatography using a diatomaceous earth packed column and the solvent system: chloroform: butanol: 0.5 *M* phosphate buffer (pH 2) (16:1:10).

EXAMPLE 9

4-Dedimethylamino-6-deoxy-7-nitrotetracycline

The methanol filtrate from example 8 was immediately adjusted to pH 1.0 with concentrated sulfuric acid. The light yellow crystalline solid, which was obtained as the sulfate salt. A purified free base was obtained by adjusting an aqueous solution of the sulfate salt (25 mg/ml) to pH 5.2 with 2 *N* sodium carbonate.

EXAMPLE 10

9-Amino-4-dedimethylamino-6-deoxytetracycline

To a solution of 300 mg of the 9-nitro compound, prepared in example 8, in 30 ml of ethanol was added 50 mg of PtO_2. The mixture was hydrogenated at atmospheric pressure until the theoretical amount of hydrogen was absorbed. The

system is flushed with nitrogen, the PtO_2 catalyst is filtered and the filtrate added dropwise to 300 ml of ether. The solid that separates is filtered and dried in a vacuum desiccator.

EXAMPLE 11

9-Acetamido-4-dedimethylamino-6-deoxytetracycline sulfate

To well stirred cold solution of 500 mg of 9-amino-4-dedimethylamino-6-deoxytetracycline sulfate, from example 10, in 2.0 ml of 1,3-dimethyl-2-imidazolidinone was added 500 mg of sodium bicarbonate followed by 0.21 ml of acetyl chloride. The mixture was stirred at room temperature for 30 minutes, filtered and the filtrate was added dropwise to 500 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 12

4-Dedimethylamino-6-deoxy-9-diazoniumtetracycline sulfate

To a solution of 0.5 g of 9-amino-4-dedimethylamino-6-deoxytetracycline sulfate, from example 10, in 10 ml of 0.1N hydrochloric acid in methanol cooled in an ice bath was added 0.5 ml of n-butyl nitrite. The solution was stirred at ice bath temperature for 30 minutes and the poured into 250 ml of ether. The solid that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 13

9-Azido-4-dedimethylamino-6-deoxytetracycline sulfate

To a solution of 0.3 mmole of 4-dedimethylamino-6-deoxy-9-diazoniumtetracycline sulfate, of example 12, 10 ml of 0.1 N methanolic hydrogen chloride was added 0.33 mmole of sodium azide. The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then poured into 200 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 14

9-Amino-8-chloro-4-dedimethylamino-6-deoxytetracycline sulfate

One gram of 9-azido-4-dedimethylamino-7-dimethylamino-6-deoxytetracycline hydrochloride, from example 13, was dissolved in 10 ml of concentrated sulfuric acid saturated with HCL at 0°C. The mixture was stirred at ice bath temperature for 1.5 hours and then slowly added dropwise to 500 ml of cold ether. The solid that separated was filtered, washed and ether and dried in a vacuum desiccator.

EXAMPLE 15

4-Dedimethylamino-6-deoxy-9-ethoxythiocarbonylthiotetracycline sulfate

A solution of 1.0 mmole of 4-dedimethylamino-6-deoxy-9-diazoniumtetracycline sulfate, from example 12, in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 16

9-Dimethylamino-4-dedimethylamino-6-deoxytetracycline sulfate

To a solution of 100 mg. of the 9-amino compound from example 10, in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml. of a 40% aqueous formaldehyde solution and 100 mg of a 10% palladium on carbon catalyst. The mixture is hydrogenated under atmospheric pressure and room temperature for 20 minutes. The catalyst was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried, yield, 98 mg.

EXAMPLE 17

7-Amino-4-dedimethylamino-6-deoxytetracycline

This compound can be made using Procedure A or B. Procedure A. To a solution of 300 mg of the 7-nitro compound, from example 1, in 30 ml of ethanol was added 50 mg of PtO_2. The mixture was hydrogenated at atmospheric pressure until the theoretical amount of hydrogen was absorbed. The system is flushed with nitrogen, the catalyst PtO_2 is filtered and the filtrate added dropwise to 300 ml of ether. The solid that separates is filtered and dried in a vacuum desiccator.

Procedure B. 1 g of 6-deoxy-4-dedimethylamino-tetracycline was dissolved in 7.6 ml THF and 10.4 ml methanesulfonic acid at -10°C. After warming the mixture to 0°C a solution of 0.86 g of dibenzyl azodicarboxylate was added and the mixture stirred for 2 hours at 0°C to yield 7-[1,2-bis(carbobenzyloxy)hydrazino]-4-dedimethylamino-6-deoxytetracycline. A solution of 1 millimole of this material in 70 ml 2-methoxyethanol, and 300 mg 10% Pd-C was hydrogenated at room temperature to give 7-amino-6-deoxy-4-dedimethylamino-tetracycline.

EXAMPLE 18

7-Amino-6-deoxy-5-hydroxy-4-dedimethylaminotetracycline

1g of 6-deoxy-5-hydroxy-4-dedimethylaminotetracycline **3** was dissolved in 7.6 ml THF and 10.4 ml methanesulfonic acid at -10°C. After warming the mixture to 0°C a solution of 0.86g dibenzyl azodicarboxylate in 0.5 ml THF was added and the mixture stirred for 2 hours at 0°C to yield 7-[1,2-bis(carbobenzyloxy)hydrazino]-4-dedimethylamino-6-deoxy-5-hydroxytetracycline. A solution of 1 millimole of this material in 70 ml 2-methoxyethanol, and 300 mg 10% Pd-C was hydrogenated at room temperature to give 7-amino-6-deoxy-5-hydroxytetracycline.

EXAMPLE 19

7-Acetamido-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate.

To well stirred cold solution of 500 mg of 7-amino-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate, from example 18, in 2.0 ml of 1,3-dimethyl-2-imidazolidinone was added 500 mg of sodium bicarbonate followed by 0.21 ml of acetyl chloride. The mixture was stirred at room temperature for 30 minutes, filtered and the filtrate was added dropwise to 500 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 20

4-Dedimethylamino-6-deoxy-5-hydroxy-7-diazoniumtetracycline hydrochloride

To a solution of 0.5 g of 7-amino-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate, from example 20, in 10 ml of 0.1N hydrochloric acid in methanol cooled in an ice bath was added 0.5 ml of n-butyl nitrite. The solution was stirred at ice bath temperature for 30 minutes and then poured into 250 ml of ether. The solid that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 21

7-Azido-4-dedimethylamino-6-deoxy-5-hydroxytetracycline

To a solution of 0.3 mmole of 4-dedimethylamino-6-deoxy-5-hydroxy-7-diazoniumtetracycline hydrochloride, from example 20, 10 ml of 0.1 N methanolic hydrogen chloride was added 0.33 mmole of sodium azide. The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then poured into 200 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 22

7-Amino-8-chloro-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate

One gram of 7-azido-4-dedimethylamino-7-dimethylamino-6-deoxy-5-hydroxytetracycline sulfate, from example 21. was dissolved in 10 ml of concentrated sulfuric acid (previously saturated with hydrogen chloride) at 0°C. The mixture was stirred at ice bath temperature for 1.5 hours and then slowly added dropwise to 500 ml of cold ether. The solid that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 23

4-Dedimethylamino-6-deoxy-5-hydroxy-7-ethoxythiocarbonylthiotetracycline

A solution of 1.0 mmole of 4-dedimethylamino-6-deoxy-5-hydroxy-7-diazoniumtetracycline hydrochloride, from example 20. in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 24

7-Dimethylamino-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate

To a solution of 100 mg of the 7-amino compound in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml of a 40% aqueous formaldehyde solution and 100 mg of a 10% palladium on carbon catalyst. The mixture is reduced with hydrogen at atmospheric pressure and room temperature for 20 minutes. The catalyst was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried, yield, 78 mg.

EXAMPLE 25

7-Diethylamino-4-dedimethylamino-5-hydroxytetracycline sulfate

To a solution of 100 mg of the 7-amino compound in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml of acetaldehyde and 100 mg of a 10% palladium on carbon catalyst. The mixture is reduced with hydrogen at atmospheric pressure at room temperature for 20 minutes. The catalyst was filtered and filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried.

EXAMPLE 26

4-Dedimethylamino-6-deoxy-7-diazoniumtetracycline hydrochloride

To a solution of 0.5 g. of 7-amino-4-dedimethylamino-6-deoxytetracycline sulfate, from example 17, in 10 ml of 0.1N hydrochloric acid in methanol cooled in an ice bath was added 0.5 ml of n-butyl nitrite. The solution was stirred at ice bath temperature for 30 minutes and then poured into 250 ml of ether. The solid that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 27

7-Azido-4-dedimethylamino-6-deoxytetracycline

To a solution of 0.3 mmole of 4-dedimethylamino-6-deoxy-7-diazoniumtetracycline hydrochloride, from example 26, 10 ml of 0.1 N methanolic hydrogen chloride was added 0.33 mmole of sodium azide. The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then poured into 200 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 28

7-Amino-8-chloro-4-dedimethylamino-6-deoxytetracycline sulfate

One gram of 7-azido-4-dedimethylamino-7-dimethylamino-6-deoxytetracycline sulfate was dissolved in 10 ml of concentrated sulfuric acid (previously saturated with hydrogen chloride) at 0°C. The mixture was stirred at ice bath temperature for 1.5 hours and then slowly added dropwise to 500 ml of cold ether. The solid that separated was filtered. washed with ether and dried in a vacuum desiccator.

EXAMPLE 29

4-Dedimethylamino-6-deoxy-7-ethoxythiocarbonylthiotetracycline

A solution of 1.0 mmole of 4-dedimethylamino-6-deoxy-7-diazoniumtetracycline hydrochloride. from example 26, in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 30

7-Dimethylamino-4-dedimethylamino-6-deoxytetracycline sulfate

To a solution of 100 mg of the 7-amino compound, from example 26, in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml of a 40% aqueous formaldehyde solution and 100 mg of a 10% palladium on carbon catalyst. The mixture is reduced with hydrogen at atmospheric pressure and room temperature for 20 minutes. The catalyst was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried.

EXAMPLE 31

9-Acetamido-8-chloro-4-dedimethylamino-7-dimethylamino-6-deoxy-6-demethyltetracycline

To well stirred cold solution of 500 mg of 9-amino-8-chloro-4-dedimethylamino-6-deoxy-6-demethyl-7-dimethyl amino tetracycline sulfate, from example 6, in 2.0 ml of 1,3-dimethyl -2-imidazolidinone was added 500 mg of sodium bicarbonate followed by 0.21 ml. of acetyl chloride. The mixture was stirred at room temperature for 30 minutes, filtered and the filtrate was added dropwise to 500 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 32

8-Chloro-4-dedimethylamino-7-dimethylamino-6-deoxy-6-demethyl-9-ethoxythiocarbonylthiotetracycline

A solution of 1.0 mmole of -8-chloro-4-dedimethylamino-6-deoxy-6-demethyl-7-dimethyl amino-9-diazoniumtetracycline hydrochloride in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 33

8-Chloro-9-dimethylamino-4-dedimethylamino-7-dimethylamino-6-deoxy-6-demethytetracycline sulfate

To a solution of 100 mg. of the 9- amino compound, from example 6, in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml of acetaldehyde and 100 mg of a 10% palladium on carbon catalyst. The mixture is reduced with hydrogen at atmospheric pressure and room temperature for 20 minutes. The catalyst was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried.

EXAMPLE 34

N-(4-methylpiperazin-1-yl) methyl-4-dedimethylamino-6-demethyl-6-deoxytetracycline

An aqueous solution of 58 mg (37%) formaldehyde (0.72 mmol) was added to a solution of 203 mg (0.49 mmol) of 4-dedimethylamino-6-demethyl-6-deoxytetracycline in 5.0 ml ethylene glycol dimethyl ether. The mixture was stirred at room temperature for 0.5 hours. 56 mg (0.56 mmol) of 1-methylpiperazine was then added and the resulting mixture was stirred overnight and refluxed for 20 minutes. The mixture was then cooled and a solid product was collected by filtration. The solid product was then washed with the solvent and dried by vacuum filtration.

EXAMPLE 35

N-(4-methylpiperazin-1-yl)methyl-4-dedimethylamino-6-demethyl-6-deoxy-9-hexanoylaminotetracycline

An aqueous solution of 49 mg 37 % formaldehyde (0.60 mmol) was added to a solution of 146 mg (0.30 mmol) of 4-dedimethylamino-6-demethyl-6-deoxy-9-hexanoylaminotetracycline in 5.0 ml ethylene glycol dimethyl ether. The mixture was stirred at room temperature for 0.5 hours. 60 mg (0.60 mmol) of 1-methylpiperazine was then added and the resulting mixture was stirred overnight and refluxed for 20 minutes. The mixture was then cooled and a solid product was collected by filtration. The solid product was then washed with the solvent and dried by vacuum filtration.

EXAMPLE 36

4-Dedimethylamino-6-demethyl-6-deoxy-9-hexanoylaminotetracycline.

1.54 g (7.2 mmol) of hexanoic anhydride and 150 mg of 10% Pd/C catalyst were added to 300 mg (0.72 mmol) of 4-dedimethylamino-6-demethyl-6-deoxytetracycline in 6.0 ml of 1,4-dioxane and 6.0 ml of methanol. The mixture was hydrogenated overnight at room temperature. The catalyst was removed by filtration

and the filtrate was concentrated under reduced pressure. The residue was dissolved in 7 ml of ethyl acetate and trituated with 50 ml of hexane to produce a solid product. The solid product was filtered and dried by vacuum filtration.

Thus, while there have been described what are presently believed to be the preferred embodiments of the present invention, those skilled in the art will realize that other and further embodiments can be made without departing from the spirit of the invention, and it is intended to include all such further modifications and changes as come within the true scope of the claims set forth herein.

EXAMPLE 37

Phototoxicity Determination

BALB/c 3T3 (CCL-163) cells were obtained from ATCC and cultured in antibiotic-free Dulbecco's Minimum Essential Medium (4.5g/l glucose)(DMEM) supplemented with L-glutamine (4mM) and 10% newborn calf serum. The working cell bank was prepared and found to be free of mycoplasma. Streptomycin sulfate (100 μg/ml) and penicillin (100 IU/ml) were added to the medium after the cells were treated with test article in 96-well plates.

Serial dilutions of the tetracycline derivatives were prepared in DMSO at concentrations 100x to final testing concentration. The CMT dilutions in DMSO were then diluted in Hanks' Balanced Salt Solution (HBSS) for application to the cells. The final DMSO concentration was 1% in treated and control cultures. For the dose range finding assay, 8 serial dilutions covered a range of 100 to 0.03 mg/ml in half log steps while the definitive assays used 6 to 8 doses prepared in quarter log steps, centered on the expected 50% toxicity point. In many cases, the dose range for treatment without UV light was different from the dose range selected with UV light. One hundred μg/ml is the highest dose recommended to prevent false negative results from UV absorption by the dosing solutions.

Controls: Each assay included both negative (solvent) and positive controls. Twelve wells of negative control cultures were used on each 96-well plate. Chlorpromazine (Sigma) was used as the positive control and was prepared and dosed like theCMTs.

Solar Simulator: A Dermalight SOL 3 solar simulator, equipped with a UVA H1 filter (320-400 nm), was adjusted to the appropriate height. Measurement of energy through the lid of a 96-well microtiter plate was carried out using a calibrated UV radiometer UVA sensor. Simulator height was adjusted to deliver 1.7 ± 0.1 m/Wcm^2 of UVA energy (resulting dose was 1J/cm^2 per 10 min.)

Phototoxicity Assay: Duplicate plates were prepared for each test material by seeding 10^4 3T3 cells per well in µl of complete medium 24 hours before treatment. Prior to treatment, the medium was removed, and the cells washed once with 125 µl prewarmed HBSS. Fifty µl of prewarmed HBSS were added to each well. Fifty µl of test article dilutions were added to the appropriate wells and the plates returned to the incubator for approximately one hour. Following the 1 hr incubation, the plates designated for the photoirritation assay were exposed (with the lid on) to 1.7 ± 0.1 mW/cm^2 UVA light for 50 ± 2 minutes at room temperature resulting in an irradiation dose of 5J/cm2. Duplicate plates designated for the cytotoxicity assay were kept in the dark room temperature for 50 ± 2 minutes. After the 50 minute exposure period the test article dilutions were decanted from the plates and the cells washed once with 125 µl HBSS. One hundred µl of medium were added to all wells and the cells incubated as above for 24 ± 1 hours.

After 24 hours of incubation, the medium was decanted and 100 µl of the Neutral Red containing media added to each well. The plates were returned to the incubator and incubated for approximately 3 hours. After 3 hours, the medium was decanted and each well rinsed once with 250 µl of HBSS. The plates were blotted to remove the HBSS and 100 µl of Neutral Red Solvent were added to each well. After a minimum of 20 minutes of incubation at room temperature (with shaking), the

absorbance at 550 nm was measured with a plate reader, using the mean of the blank outer wells as the reference. Relative survival was obtained by comparing the amount of neutral red taken by test article and positive control treated groups to the neutral red taken up by the negative group on the same plate. IC_{50} values for both the UVA exposed and non-exposed groups were determined whenever possible. One dose range finding and at least two definitive trails were performed on each CMT.

Determination of Phototoxicity: Phototoxicity of the tetracycline derivatives can be measured by its photoinhibition factor (PIF). The PIF was determined by comparing the IC_{50} without UVA [IC_{50}(-UVA)] with the IC_{50} with UVA [IC_{50}(+UVA)]:

$$PIF = \frac{IC_{50}(-UVA)}{IC_{50}(+UVA)}$$

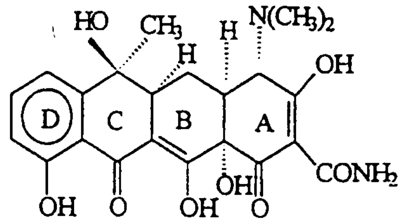
If both IC_{50} values can be determined, the cut off value of the factor to discriminate between phototoxicants and non-phototoxicants is a factor of 5: A factor greater than 5 is indicative of phototoxic potential of the test material.

If a chemical is only cytotoxic +UVA and not cytotoxic when tested -UVA, the factor can not be calculated, although it may have clear result indicating some level of phototoxic potential. In this case, a ">PIF" can be calculated and the highest testable dose (-UVA) will be used for calculation of the ">PIF".

$$>PIF = \frac{\text{maximum dose } (-UVA)}{IC_{50}(+UVA)}$$

If both, IC_{50}(-UVA) and IC_{50}(+UVA) cannot be calculated because the chemical does not show cytotoxicty (50% reduction in viability) up to the highest dose tested, this would indicate a lack of phototoxic potential.

INDEX OF STRUCTURES



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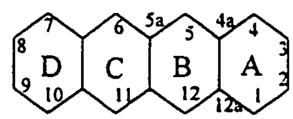

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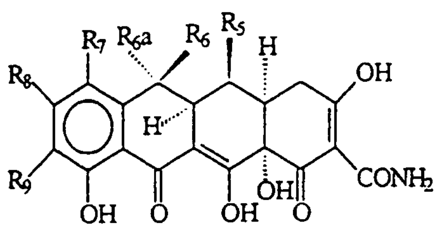

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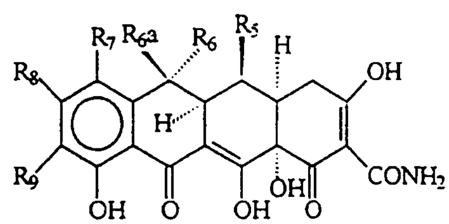

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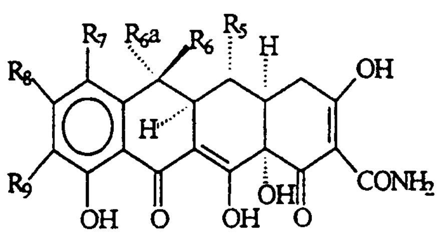

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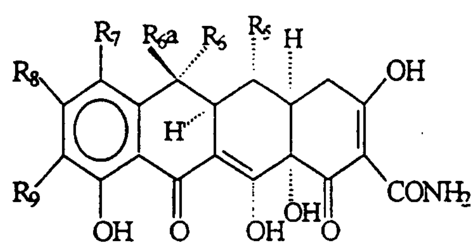

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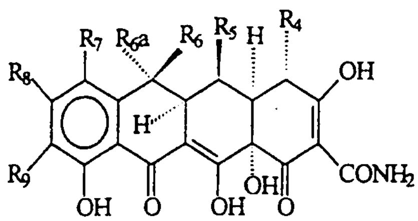

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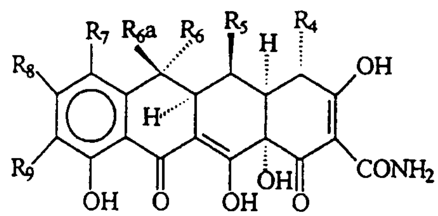

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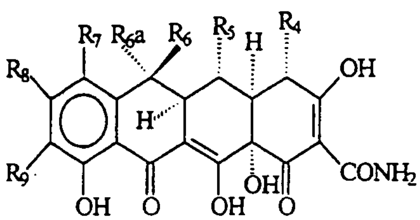

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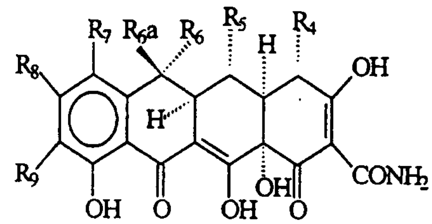

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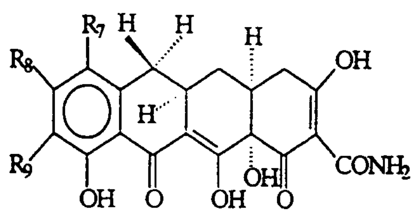

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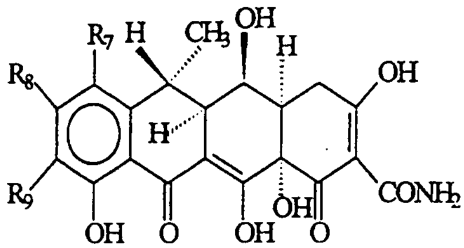

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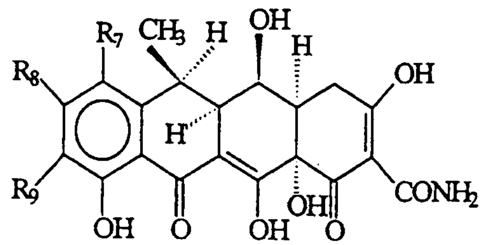

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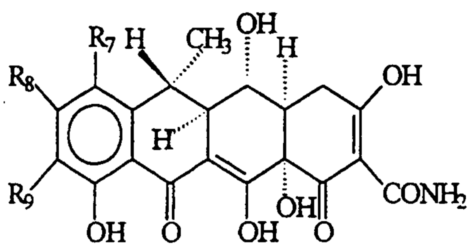

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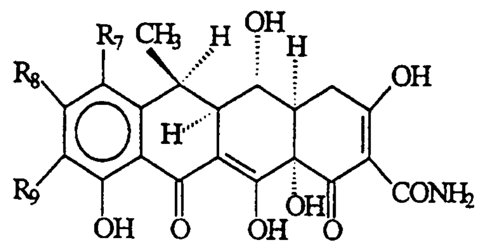

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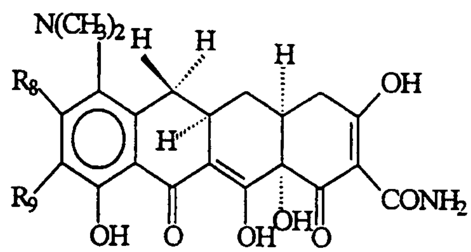

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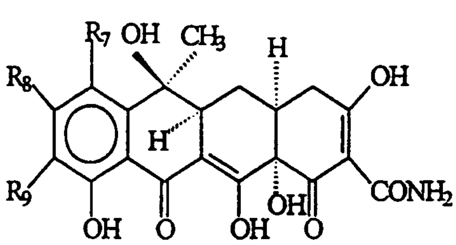

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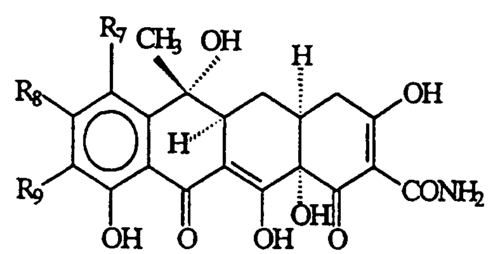

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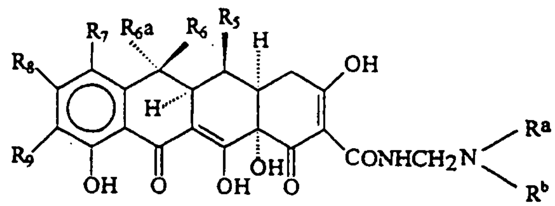

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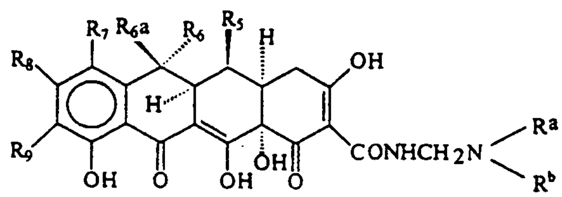

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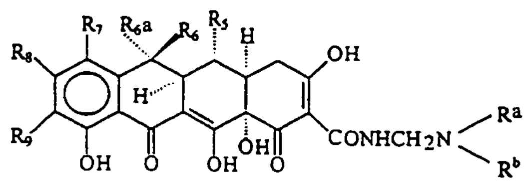

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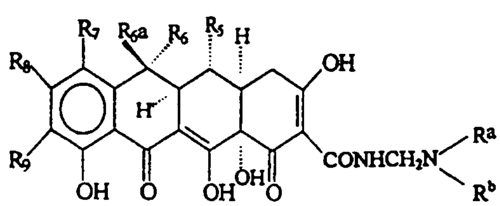

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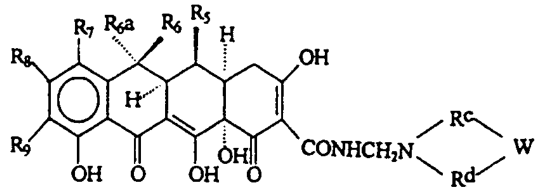

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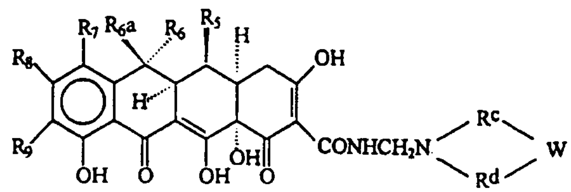

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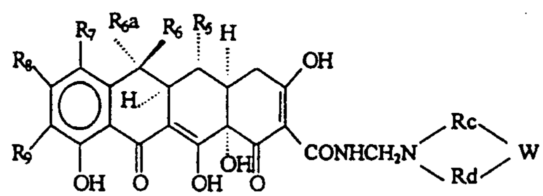

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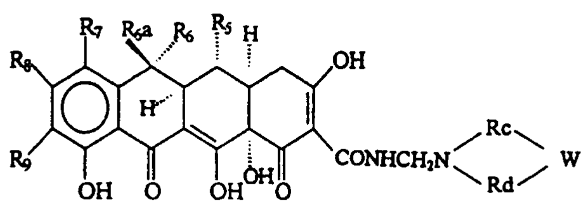
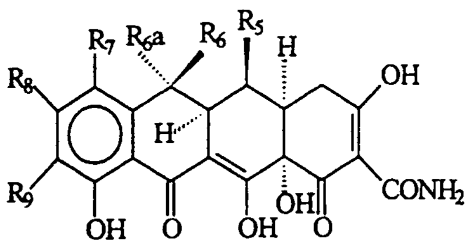


Structure Z

WHAT IS CLAIMED IS:

1. A tetracycline compound of the formulae:



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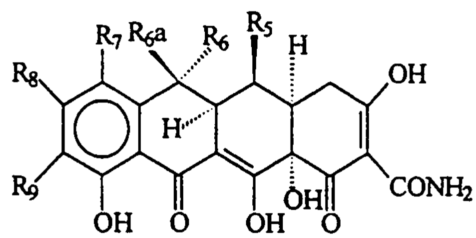

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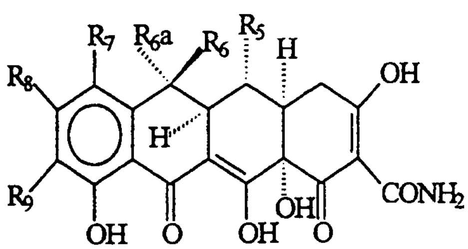
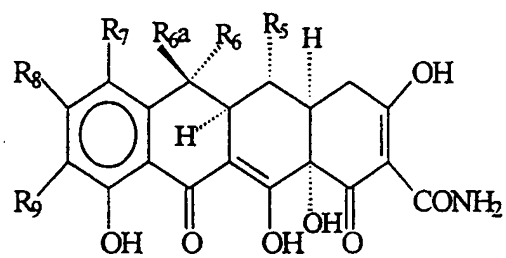


Structure E

or



Structure F

wherein:

R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl;

R6-a is selected from the group consisting of hydrogen and methyl;

R6 and R5 are selected from the group consisting of hydrogen and hydroxyl;

R8 is selected from the group consisting of hydrogen and halogen;

R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, diazonium, di(lower alkyl)amino and $RCH(NH_2)CO$;

R is hydrogen or lower alkyl; and

pharmaceutically acceptable and unacceptable salts thereof; with the following provisos:

when either R7 and R9 are hydrogen then R8 must be halogen; and

when R6-a, R6, R5 and R9 are all hydrogen and R7 is hydrogen, amino, nitro, halogen, dimethylamino or diethylamino, then R8 must be halogen; and

when R6-a is methyl, R6 and R9 are both hydrogen, R5 is hydroxyl, and R7 is hydrogen, amino, nitro, halogen or diethylamino, then R8 is halogen; and

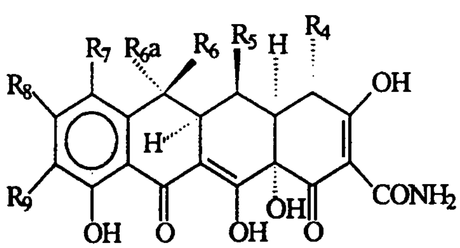
when R6-a is methyl, R6 is hydroxyl, R5, R7 and R9 are all hydrogen, then R8 must be halogen; and

when R6-a, R6 and R5 are all hydrogen, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and

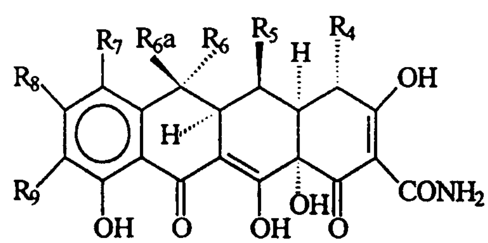
when R6-a is methyl, R6 is hydrogen, R5 is hydroxyl, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and

when R6-a is methyl, R6, R5 and R9 are all hydrogen and R7 is cyano, then R8 must be halogen.

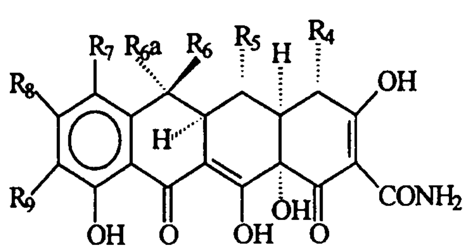
2. A tetracycline compound of the formulae:



Structure G

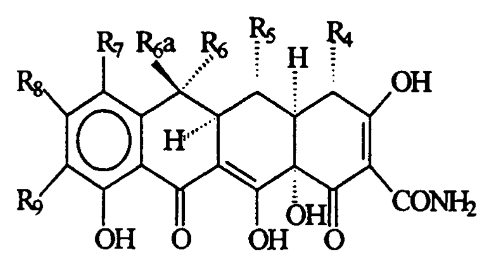


Structure H



Structure I

or



Structure J

wherein:

R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, and di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl;

R6-a is selected from the group consisting of hydrogen and methyl;

R6 and R5 are selected from the group consisting of hydrogen and hydroxyl;

R4 is selected from the group consisting of NOH, N-NH-A, and NH-A, where A is a lower alkyl group;

R8 is selected from the group consisting of hydrogen and halogen;

R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio. mono(lower alkyl) amino, halogen, di(lower alkyl)amino and $RCH(NH_2)CO$;

R is hydrogen or lower alkyl; and

pharmaceutically acceptable and unacceptable salts thereof; with the following provisos:

when R4 is NOH, N-NH-alkyl or NH-alkyl and R7, R6-a, R6, R5, and R9 are all hydrogen, then R8 must be halogen; and

when R4 is NOH, R6-a is methyl, R6 is hydrogen or hydroxyl, R7 is halogen, R5 and R9 are both hydrogen, then R8 must be halogen; and

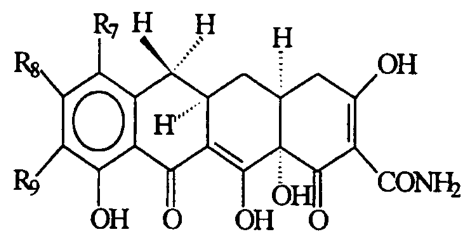
when R4 is N-NH-alkyl, R6-a is methyl, R6 is hydroxyl and R7, R5, R9 are all hydrogen, then R8 must be halogen; and

when R4 is NH-alkyl, R6-a, R6, R5 and R9 are all hydrogen, R7 is hydrogen, amino, mono(lower alkyl)amino, halogen, di(lower alkyl)amino or hydroxyl, then R8 must be halogen; and

when R4 is NH-alkyl, R6-a is methyl, R6 and R9 are both hydrogen, R5 is hydroxyl, and R7 is mono(lower alkyl)amino or di(lower alkyl)amino, then R8 must be halogen; and

when R4 is NH-alkyl, R6-a is methyl, R6 is hydroxy or hydrogen and R7, R5, and R9 are all be hydrogen, then R8 must be halogen.

3. A 4-dedimethylamino tetracycline compound selected from:

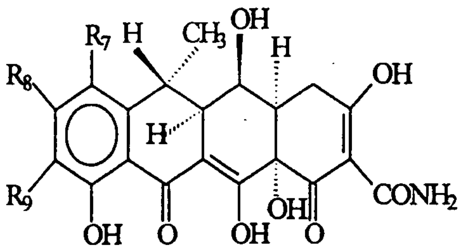


Structure K

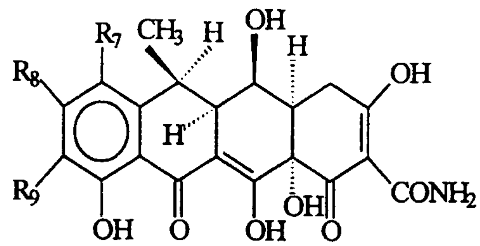
wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	acylamino
dimethylamino	hydrogen	diazonium
dimethylamino	chloro	amino
hydrogen	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkylamino	chloro	amino
nitro	chloro	amino
dimethylamino	chloro	acylamino
dimethylamino	chloro	dimethylamino
hydrogen	hydrogen	dimethylamino
dimethylamino	hydrogen	hydrogen

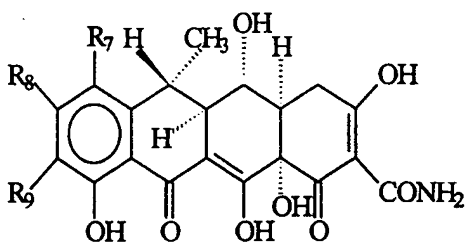
and



Structure L

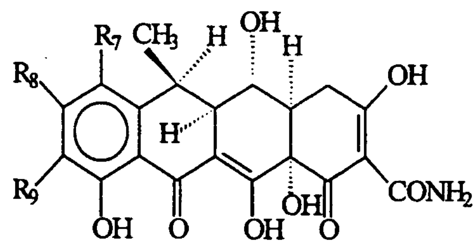


Structure M



Structure N

or



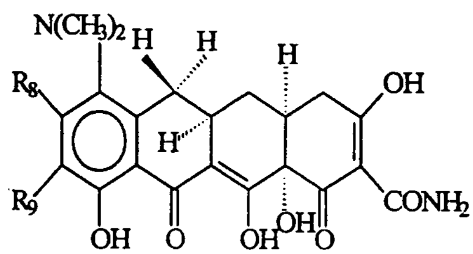
Structure O

wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	acylamino
hydrogen	hydrogen	diazonium
hydrogen	hydrogen	dimethylamino

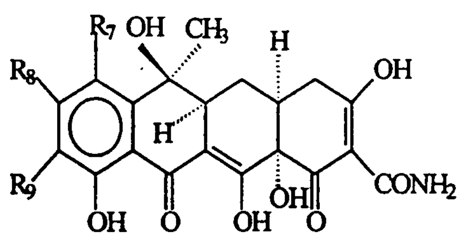
diazonium	hydrogen	hydrogen
ethoxythiocarbonylthio	hydrogen	hydrogen
dimethylamino	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
hydrogen	chloro	amino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkylamino	chloro	amino
nitro	chloro	amino

and



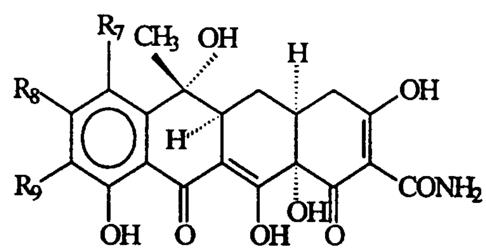
Structure P

wherein: R8 is hydrogen or halogen and R9 is selected from the group consisting of nitro, (N,N-dimethyl)glycylamino, and ethoxythiocarbonylthio; and



Structure Q

or



Structure R

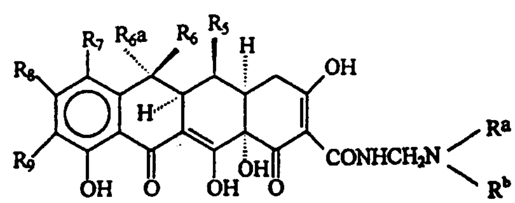
wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
amino	hydrogen	hydrogen
nitro	hydrogen	hydrogen
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino

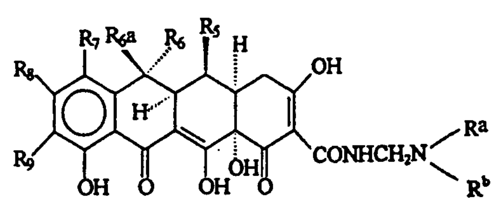
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
bromo	hydrogen	hydrogen
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
diethylamino	hydrogen	hydrogen
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	methylamino
dimethylamino	hydrogen	acylamino
dimethylamino	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
hydrogen	chloro	amino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkylamino	chloro	amino
nitro	chloro	amino

and pharmaceutically acceptable and unacceptable salts thereof.

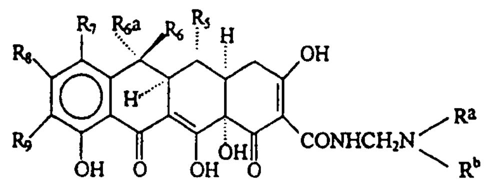
4. A tetracycline compound of the formulae:



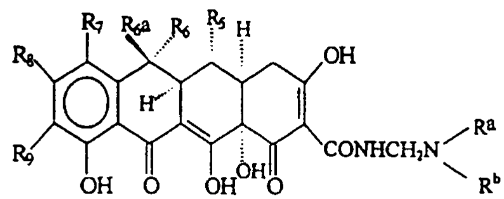
Structure S



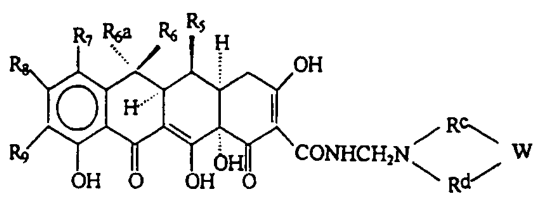
Structure T



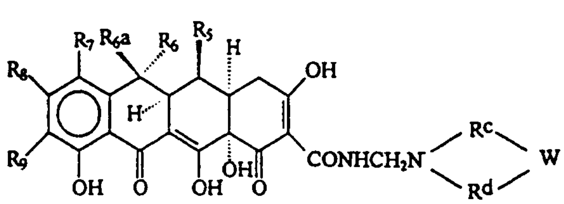
Structure U



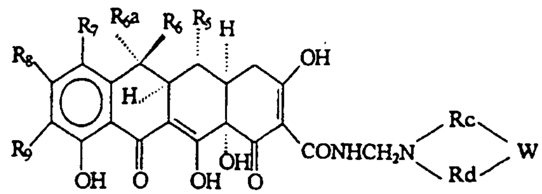
Structure V



Structure W

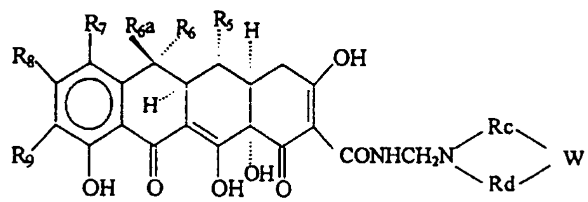


Structure X



Structure Y

or



Structure Z

wherein:

R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl;

R6-a is selected from the group consisting of hydrogen and methyl;

R6 and R5 are selected from the group consisting of hydrogen and hydroxyl;

R8 is selected from the group consisting of hydrogen and halogen;

R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, diazonium, di(lower alkyl)amino and $RCH(NH_2)CO$;

R is hydrogen or lower alkyl;

R^a and R^b are selected from the group consisting of hydrogen, methyl, ethyl, n-propyl and 1-methylethyl with the proviso that R^a and R^b cannot both be hydrogen;

R^c and R^d are, independently, $(CH_2)_nCHR^e$ wherein n is 0 or 1 and R^e is selected from the group consisting of hydrogen, alkyl, hydroxy, lower(C_1-C_3) alkoxy, amino, or nitro; and,

W is selected from the group consisting of $(CHR^e)_m$ wherein m is 0-3 and said R^e is as above, NH, $N(C_1-C_3)$ straight chained or branched alkyl, O, S and $N(C_1-C_4)$ straight chain or branched alkoxy; and,

pharmaceutically acceptable and unacceptable salts thereof.

5. A method according to claim 4 with the following provisos:

when either R7 and R9 are hydrogen then R8 must be halogen; and

when R6-a, R6, R5 and R9 are all hydrogen and R7 is hydrogen, amino, nitro, halogen, dimethylamino or diethylamino, then R8 must be halogen; and

when R6-a is methyl, R6 and R9 are both hydrogen, R5 is hydroxyl, and R7 is hydrogen, amino, nitro, halogen or diethylamino, then R8 is halogen; and

when R6-a is methyl, R6 is hydroxyl, R5, R7 and R9 are all hydrogen, then R8 must be halogen; and

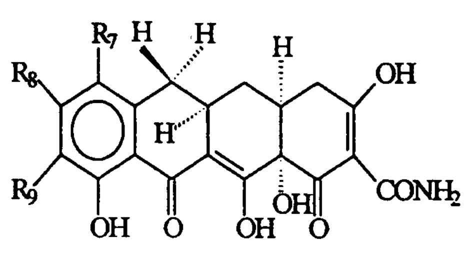
when R6-a, R6 and R5 are all hydrogen, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and

when R6-a is methyl, R6 is hydrogen, R5 is hydroxyl, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and

when R6-a is methyl, R6, R5 and R9 are all hydrogen and R7 is cyano, then R8 must be halogen.

6. A tetracycline compound according to Claim 1 wherein the PIF is about 1.

7. A tetracycline compound according to Claim 6 selected from the group consisting of:

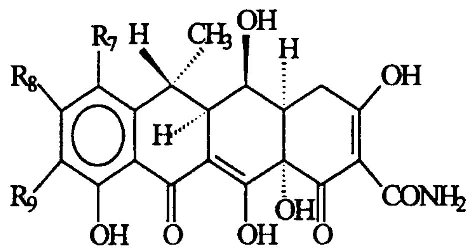


Structure K

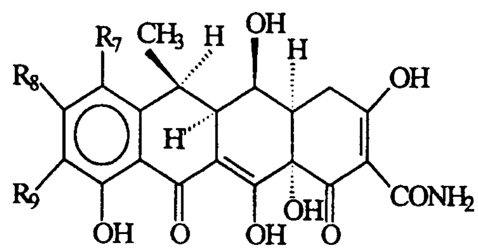
wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	amino
hydrogen	hydrogen	palmitamide

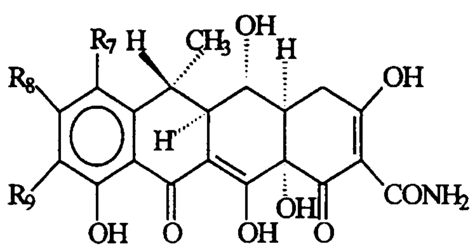
and



Structure L

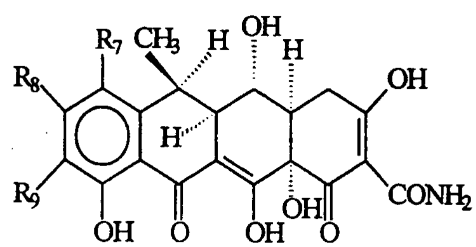


Structure M



Structure N

or

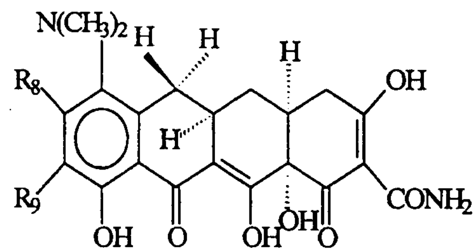


Structure O

wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	acetamido
hydrogen	hydrogen	dimethylaminoacetamido
hydrogen	hydrogen	nitro
hydrogen	hydrogen	amino

and

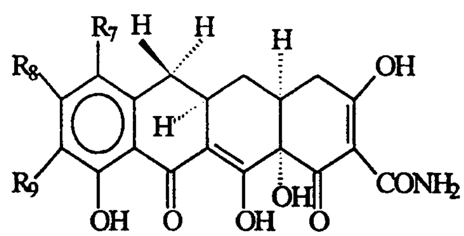


Structure P

wherein: and R7, R8, and R9 taken together are, respectively, hydrogen, hydrogen and nitro.

8. A tetracycline compound according to Claim 1 wherein the PIF has a value between 1 and 2.

9. A tetracycline compound according to Claim 8 having general forumula:



Structure K

wherein: R7, R8, and R9 taken together are, respectively, hydrogen, hydrogen and dimethylamino.

10. A method for treating a mammal suffering from a condition that benefits from a non-antimicrobial dose of a tetracycline compound, said condition

being characterized by excessive collagen destruction, excessive MMP enzyme activity, excessive TNF activity, excessive nitric oxide activity, excessive IL-1 activity, excessive elastase activity, excessive loss of bone density, excessive protein degradation, excessive muscle wasting, excessive glycosylation of collagen, excessive COX-2 activity, insufficient bone protein synthesis, insufficient interleukin-10 production or excessive phospholipase A_2 activity the method comprising administering to the mammal an effective amount of a tetracycline compound according to claim 1, 2, 3 or 4.

11. A method according to claim 10, wherein said condition is abdominal aortic aneurysm, ulceration of the cornea, periodontal disease, diabetes, diabetes mellitus, scleroderma, progeria, lung disease, cancer, graft versus host disease, disease of depressed bone marrow function, thrombocytopenia, prosthetic joint loosening, spondyloarthropathies, osteoporosis, Paget's disease, autoimmune disease, systemic lupus erythematosus, acute or chronic inflammatory condition, renal disease or connective tissue disease.

12. A method according to claim 11, wherein said acute or chronic inflammatory condition is inflammatory bowel disease, arthritis, osteoarthritis, rheumatoid arthritis, pancreatitis, nephritis, glomerulonephritis, sepsis, septic shock, lipopolysaccharide endotoxin shock, multisystem organ failure or psoriasis.

13. A method according to claim 11, wherein said lung disease is ARDS, cystic fibrosis, emphysema or acute lung injury resulting from inhalation of toxicants.

14. A method according to claim 11, wherein said renal disease is chronic renal failure, acute renal failure, nephritis or glomerulonephritis.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27304

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, WEST, CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2,786,077 A (STEPHENS, JR.) 19 March 1957, column 1, lines 40-70.	1-3 and 5-14
Y	US 5,122,519 A (RITTER) 16 June 1992, column 3, lines 10-60.	1-3 and 5-14
Y	US 3,849,493 A (CONOVER et al) 19 November 1974, column 2, lines 50-70.	1-3 and 5-14
Y	US 3,345,370 A (ESSE et al) 03 October 1967, columns 1 and 2.	1-3 and 5-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 MARCH 2000	04 APR 2000

Name and mailing address of the ISA/US
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Washington, D.C. 20231
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Authorized officer
SHAILENDRA KUMAR
Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27304

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

A61K 31/165, 31/5375, 31/40, 31/495; C07C 237/32, 237/34, 237/36, 237/38; C07D 295/00, 207/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/152, 238.2, 427, 255; 552/202; 544/168, 169, 380; 548/528

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

514/152, 238.2, 427, 255; 552/202; 544/168, 169, 380; 548/528